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Antifibrotic therapies for metabolic dysfunction-associated steatotic liver disease

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Summary

Metabolic dysfunction-associated steatotic liver disease (MASLD) affects more than a quarter of the adult population worldwide. MASLD can progress to metabolic dysfunction-associated steatohepatitis (MASH), which is associated with increased risk of progression to liver fibrosis, cirrhosis and hepatocellular carcinoma, as well as cardiovascular complications. The pathogenesis of MASLD is complex and initiated by altered metabolic signalling circuits between the adipose tissue, muscle, gut and liver. Liver fibrosis is largely driven by the crosstalk of steatotic hepatocytes with macrophages and hepatic stellate cells and constitutes the primary determinant of outcomes in MASLD. Therefore, fibrosis regression is a key therapeutic goal for MASH therapies. Here, we review therapeutic strategies that directly or indirectly reduce liver fibrosis and discuss novel therapeutic concepts. Among these, the targeting of hepatocytes and metabolism have yielded fibrosis reduction in clinical trials and led to the first FDA-approved therapy for MASH. However, these therapies reduce fibrosis only in a subset of patients and have not yet shown benefits beyond the F2-F3 fibrosis stage. Direct antifibrotics and macrophage-based therapies may be more suitable for advanced stages of MASH, but are still in the developmental stage. The arsenal of therapies for MASLD is rapidly expanding and includes macrophage transplantation, hepatocyte-specific oligonucleotides, as well as CAR T cell-based therapies. Integrating these novel therapeutic concepts into stage-specific and/or combination therapies targeting divergent pathogenic mechanisms and cell types is the focus of ongoing research, which may lead to fibrosis reduction in a higher percentage of patients with MASH.

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Introduction

Metabolic dysfunction-associated liver disease (MASLD) is closely associated with obesity and type 2 diabetes mellitus.[1,2] With nearly 2 billion adults worldwide and 75% of adult Americans being overweight or obese,[3] the rates of MASLD have dramatically increased, affecting about 30% of adults worldwide.[4,5] Alarmingly, the rates of obesity and MASLD among adolescents have also increased steadily,[3,6,7] leading to an earlier onset and longer duration of MASLD, which is likely to increase the risk of developing MASLD-associated complications in the lifetime of affected individuals. MASLD can progress to a more aggressive form, termed metabolic dysfunction-associated steatohepatitis (MASH), which is characterised by inflammation and elevated risk for disease progression toward fibrosis, cirrhosis and the development of hepatocellular carcinoma (HCC).[1,2]

Liver fibrosis represents the primary determinant of mortality in patients with MASLD, and is associated with increased liver-related events, including the development of HCC, as well as cardiovascular outcomes.[8–12] The accurate and sensitive identification of patients with MASH and liver fibrosis remains challenging, as liver biopsy is rarely performed nowadays. Clinically, liver fibrosis is routinely assessed via non-invasive tests, including imaging approaches that include vibration-controlled transient elastography (*e.g.* FibroScan), magnetic resonance elastography, corrected T1 weighted imaging, or serologic marker-based scores, such as the Fibrosis-4 index and enhanced liver fibrosis (ELF) test.[13–16] However, many non-invasive tests perform best in detecting advanced fibrosis stages (>F3).[14,15,17] Although pathologic scoring of fibrosis by stepwise scoring systems has been the basis for assessing severity and changes in fibrosis,[18] it is increasingly clear that digital methodologies are far more accurate and quantitative, not only in assessing fibrosis, but also other structural and cellular features of disease (reviewed in Refs.[19,20]). While their advantages are incontrovertible, they have not yet been approved by regulatory agencies as alternative endpoints for clinical trials.

Since recent therapeutic concepts embrace earlier treatment, more refined strategies have been developed to reliably quantify fibrosis at earlier stages by combining imaging and serologic tests such as FibroScan plus aspartate aminotransferase (FAST) score, the MRI plus AST (MAST) score and the MRE plus FIB-4 (MEFIB) index.[14] Newer serological tests (*e.g.* NIS2+) aim at identifying "at-risk MASH" based on steatohepatitis activity and/or relevant fibrosis.[21] For further details on current tests and future developments, we refer to recent reviews on this topic.[13,14,22,23]

MASLD is a systemic disease involving crosstalk between the liver, adipose tissue, muscle and gut.[1,24] Whereas the development of steatosis in early MASLD stages is driven by systemic alterations of lipid and glucose metabolism in multiple organs, the progression to MASH fibrosis and MASH cirrhosis

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Keypoints

- Liver fibrosis is the key determinant of outcomes in patients with metabolic dysfunction-associated steatotic liver disease (MASLD).
- In MASLD, liver fibrosis is driven by crosstalk between different cell types, including hepatocytes, macrophages, immune cells and hepatic stellate cells (HSCs).
- To date, the only strategies shown to reduce fibrosis in MASLD have been indirect, *i.e.* targeting hepatocytes and metabolism.
- The success of hepatocyte- and metabolism-targeting drugs has mostly been validated in patients with MASLD and F2-F3 fibrosis.
- Direct antifibrotic drugs, targeting HSCs, extracellular matrix production or degradation, would be desirable for MASLD patients with advanced fibrosis or cirrhosis. However, so far, direct antifibrotics have not achieved a strong reduction of fibrosis in clinical trials and/or their development has been terminated.
- A wide range of direct antifibrotics remain under investigation, including cell therapies.
- Drugs targeting macrophages, for example to increase restorative macrophages, may reduce fibrosis or promote fibrosis resolution, and are currently under investigation.
- Understanding different cell states and cell-cell communication in MASLD will likely lead to new antifibrotic and regenerative therapies.

is, in large part, a consequence of the crosstalk among different cell types within the liver.[25,26] Key players in this intrahepatic crosstalk include hepatocytes, hepatic stellate cells (HSCs), liver sinusoidal endothelial cells (LSECs) and specific subsets of immune cells.[27] While the dynamics of this cellular crosstalk have not been fully unravelled, these interactions are often bi- or multidirectional, involving multiple cell types that closely interact and form cellular modules rather than single cell types that act as isolated disease drivers.[25,28] For example, immune cell recruitment and subsequent inflammation appear to be a consequence of metabolic hepatocyte stress and injury, but inflammatory cells may also drive hepatocyte steatosis and injury.[29,30] Likewise, HSC activation is a consequence of hepatocyte injury but the loss of hepatoprotective factors in activated HSCs may also contribute to increased hepatocyte injury.[31,32] The healthy liver contains negative feedback loops that preserve homeostasis,[31,32] but these are replaced by feed-forward loops in the injured liver, amplifying steatosis, injury and fibrogenesis.[33–36] In addition to MASLD-promoting diets and lifestyle, there are also a wide range of genetic factors that influence its development and progression.[1,2,37,38] Thus, treatment options for liver fibrosis span a wide range of cellular targets and interventions. Because of the multicellular signalling circuits in MASH, targeting one cell type in the liver may impact many other cell types.[39]

Herein, we review current concepts for antifibrotic therapies in MASLD. While HSCs represent the primary fibrogenic cell type of the liver, there are currently no approved direct antifibrotic treatments for MASLD. However, targeting hepatocyte metabolism has proven to be an effective approach for MASLD and may indirectly improve liver fibrosis (Fig. 1). Moreover, macrophages not only play a key role in activating HSCs but also contribute to the resolution of liver fibrosis, and are currently being investigated as fibrosis-resolving therapies in clinical trials (Fig. 1).[26,40–42,43] We review the underlying pathophysiology and key players, stage-specific therapies and the combination of direct and indirect antifibrotic therapies. Furthermore, we highlight emerging therapeutic concepts, including hepatocyte-directed, RNA-based therapies, as well as those that harness the restorative properties of macrophage and HSC subpopulations to restore liver architecture and function.

HSC cell states and functions in MASLD

HSCs are the primary fibrogenic cell type in the liver and, hence, one of the key targets for direct antifibrotic therapies. However, the few clinical trials testing direct antifibrotic therapies targeting HSCs and/or fibrogenesis have not been successful, suggesting that more refined strategies may be required. Recent studies suggest distinct HSC states differentially impact homeostasis, liver function, fibrosis and disease progression.[39] Understanding these distinct functions and cell states will be important for the development of novel antifibrotic therapies, with the focus being on targeting pathogenic HSC states and mediators linked to fibrosis and inflammation, while restoring HSC states and mediators associated with homeostasis, hepatoprotection and fibrosis resolution (Fig. 2) (see "Direct antifibrotic therapies targeting HSCs in MASH – Emerging strategies").

Quiescent HSCs promote liver homeostasis

In the healthy liver, HSCs maintain a quiescent and non-proliferative phenotype. Quiescent HSCs (qHSCs) are the main reservoir for vitamin A, which store 50-80% of the body's total vitamin A within cytoplasmic lipid droplets in the form of retinyl esters.[44] Retinyl ester storage in HSCs is mediated by lecithin retinol acyltransferase, which is highly enriched in HSCs.[44] In addition to maintaining systemic levels of retinoids, lecithin retinol acyltransferase has a role in promoting liver regeneration after 70% partial hepatectomy.[45] Beyond the storage of vitamin A, increasing evidence suggests that qHSCs are also responsible for maintaining key aspects of liver homeostasis, including the metabolic functions of hepatocytes.[32,39,46–48] Their position within the space of Disse and long cellular projections allow HSCs to maintain close contact with liver sinusoidal endothelial cells (LSECs), Kupffer cells (KCs) and hepatocytes. Wake and colleagues proposed that individual HSCs can contact up to twenty hepatocytes and several LSECs in a multicellular unit termed a "stellon".[49] This concept is further supported by single-cell RNA-seq analyses, indicating that HSCs are among the most interactive of cell types in the liver.[50] Along this line, qHSCs are enriched in several cytokines and growth factors through which they can maintain crosstalk with hepatocytes, KCs and LSECs.[28,31,50,51]

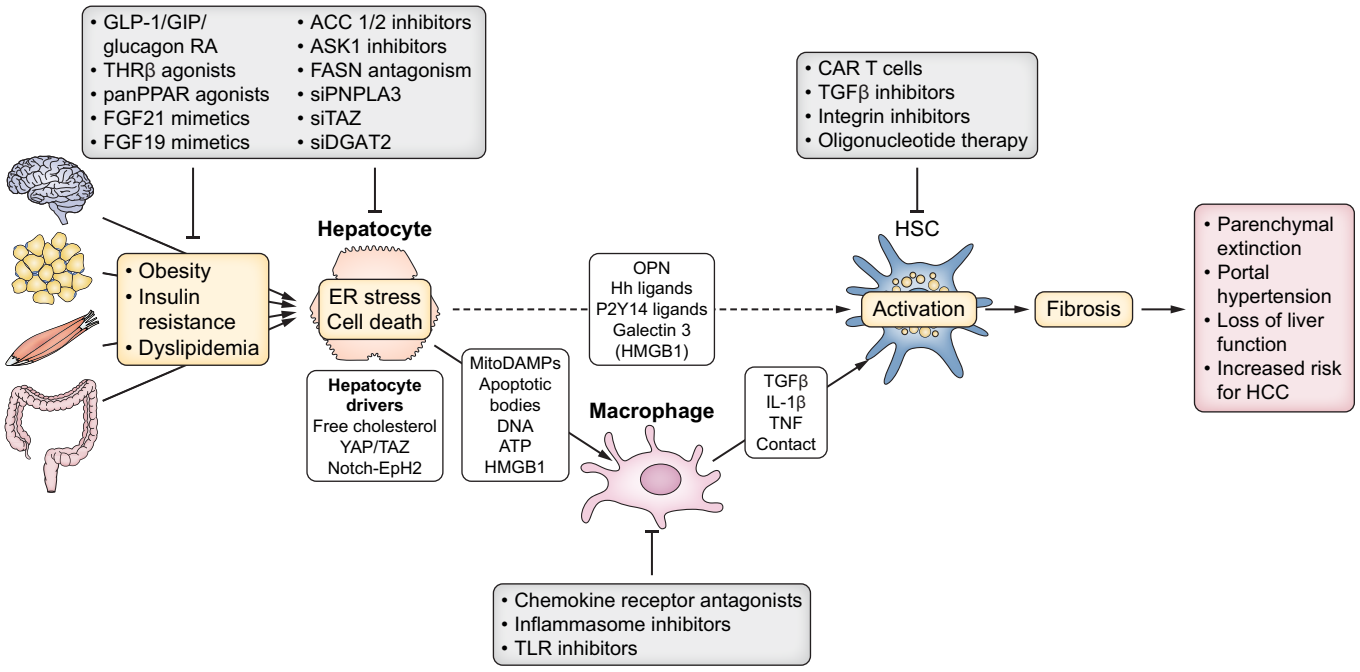

Fig. 1. Therapeutic interruption of the cellular crosstalk that promotes liver fibrosis in MASLD. Obesity, insulin resistance and dyslipidaemia increase hepatocyte steatosis, ER stress and cell death as well as activation of the YAP/TAZ pathway and NOTCH pathways. TAZ- and NOTCH-driven secretion of hedgehog ligands and OPN, as well as secretion of galectin 3 and DAMPs like P2Y14 ligand UDP-glucose may directly promote the activation of HSCs. Apoptotic bodies, mitochondrial DAMPs and other DAMPs, such as DNA and HMGB1, activate macrophages, which in turn secrete TGFβ, IL-1β and TNF to promote HSC activation and survival in MASH. Together, this may result in progressive liver fibrosis with parenchymal extinction and loss of liver function as well as the development of portal hypertension and increased risk for the development of HCC. Several therapies that target metabolism and hepatocytes, including GLP-1/GIP/glucagon RA, THRβ agonists, pan-PPAR agonists, FGF21 mimetics, as well as a large number of drugs still under investigation, may improve hepatocyte steatosis, stress, cell death and mediators that promote HSC and macrophage activation and, thereby, reverse liver fibrosis. Targeting macrophages (*e.g.* via chemokine receptor antagonism, inflammasome inhibitors and TLR inhibitors) and HSCs (*e.g.* via CAR T cells, TGFβ inhibitors, integrin inhibitors or oligonucleotide therapy) has not yet been proven to reverse liver fibrosis in patients with MASLD but remains promising. CAR, chimeric antigen receptor; DAMPs, damage-associated molecular patterns; ER, endoplasmic reticulum; HCC, hepatocellular carcinoma; Hh, hedgehog; HMGB1, high molecular group box 1; OPN, osteopontin; TGFβ, transforming growth factor β; TLR, Toll-like receptor; TNF, tumour necrosis factor.

Key qHSC-enriched mediators include hepatocyte growth factor (HGF), R-spondin 3 (RSPO3), neurotrophin-3 (NTF3) and bone morphogenetic protein 9 and 10 (BMP9, BMP10)[32,46,48] (Fig. 2). A central function of qHSCs, mediated by RSPO3, is the regulation of liver zonation.[32] HSCs show a pericentral to periportal gradient of RSPO3 expression, which is required for the activation of WNT/β-catenin signalling in pericentral to mid-lobular hepatocytes, and contributes to the zonal expression of characteristic WNT-regulated genes such as *Cyp2e1* and *Cyp1a2*.[32,52] Moreover, HSC-expressed RSPO3 is required for efficient liver regeneration, consistent with the key role of the WNT/β-catenin pathway in hepatocyte proliferation and liver regeneration.[32] BMP9 and BMP10 are additional growth factors that are enriched in qHSCs and exert critical functions in liver homeostasis.[46] HSC-derived BMP9 and BMP10, which often act in tandem as they are the only known ligands for Alk1,[53] provide signals that maintain endothelial cell and KC identity.[46] Furthermore, via their effects on LSECs, they also affect liver zonation and regulate iron metabolism.[46]

HGF is a growth factor enriched in qHSCs that does not have an established role in liver homeostasis, apart from protecting hepatocytes from injury in the healthy liver. Although HGF is also a complete mitogen for hepatocytes, its deletion in HSCs does not affect liver regeneration, as LSECs also express HGF.[31] NTF3 is also enriched in qHSCs and may drive liver regeneration, as shown by the mitogenic effects of recombinant NTF3 *in vitro* and increased hepatocyte proliferation following NTF3 overexpression *in vivo*.[48,54] In summary, qHSCs express several growth factors that maintain liver homeostasis and zonation, protect the liver from injury and promote regeneration.[39] These beneficial functions of qHSCs are progressively lost as MASLD progresses, as detailed in the following section.

Activated HSCs promote fibrogenesis and lose homeostatic functions

Activated HSCs are the primary collagen-producing cells of the liver in a wide range of diseases, including MASLD.[55–57] In response to liver injury, qHSCs undergo a well-characterised activation process, and transdifferentiate into extracellular matrix-producing, contractile myofibroblasts.[58] Activated HSCs display profound morphologic and transcriptomic alterations, including the loss of their characteristic lipid droplets, a more myofibroblastic spindle-like shape, and the acquisition of proliferative, migrative, chemotactic and contractile capabilities.[59] The differentiation from qHSCs to activated HSCs is mediated by a wide range of signalling molecules, including transforming growth factor-β (TGFβ), the most potent activator of HSCs, as well as platelet-derived growth factor (PDGF) and connective tissue growth factor (CTGF or CCN2), which drive the expansion of HSCs and their migration. Additional mediators promoting

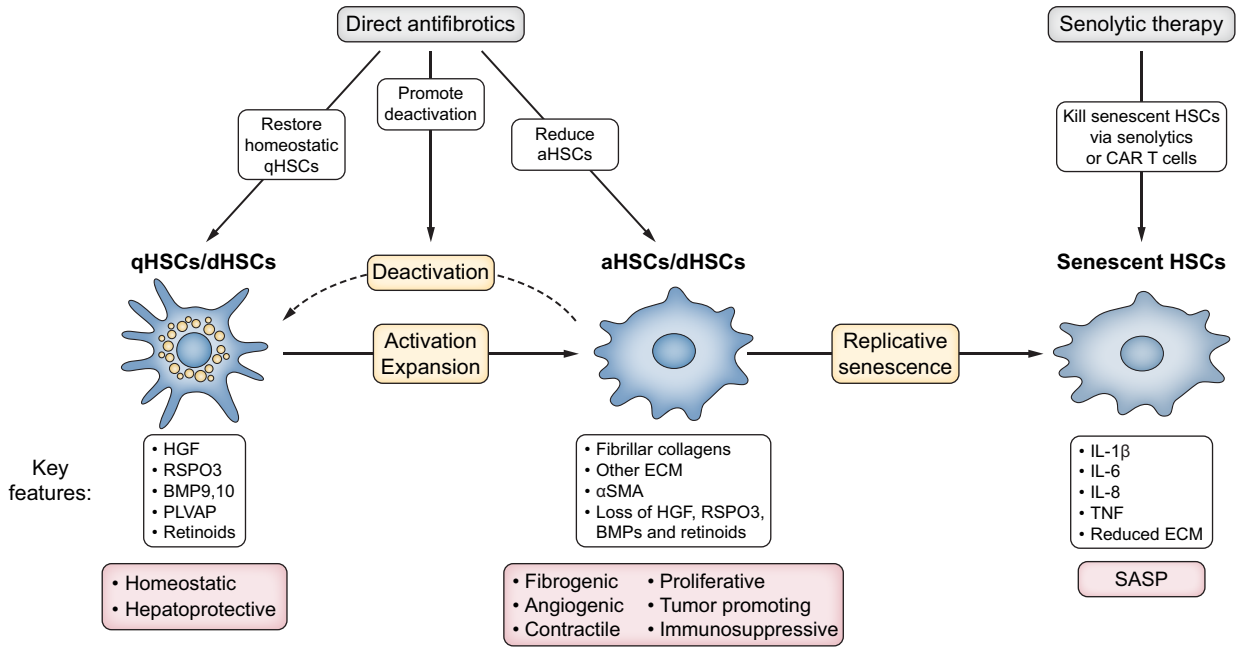

Fig. 2. Targeting distinct HSC states in MASLD. In MASLD, HSCs exist in four main states: quiescent, activated, deactivated, and senescent. qHSCs are characterised by their homeostatic and hepatoprotective properties, expressing mediators such as HGF, RSPO3 and BMPs. Following MASH-induced liver injury, HSCs activate and proliferate and acquire fibrogenic, angiogenic, contractile, immunosuppressive and tumour-promoting properties through the expression of fibrillar collagens, non-collagenous ECM, αSMA and the loss of hepatoprotective mediators, HGF, RSPO3 and BMPs. With improved MASLD, HSCs may deactivate (dHSCs) and return to a near-quiescent state. In progressive MASLD, HSCs may undergo senescence, characterised by the "senescence-associated secretory phenotype" (SASP), with increased IL-1β, IL-6, IL-8, and TNF expression as well as lower ECM expression. HSC-directed therapeutic strategies in MASLD include the restoration of a healthy HSC balance by reducing pathogenic aHSCs and increasing protective qHSCs/dHSCs; as well as by eliminating sHSCs. aHSCs, activated HSCs; BMP, bone morphogenetic protein; dHSCs, deactivated HSCs; ECM, extracellular matrix; HGF, hepatocyte growth factor; MASH, metabolic dysfunction-associated steatohepatitis; MASLD, metabolic dysfunction-associated steatotic liver disease; qHSCs, quiescent HSCs; PLVAP, plasmalemma vesicle-associated protein; RSPO3, R-spondin 3; SASP, senescence-associated secretory phenotype; sHSCs, senescent HSCs.

HSC activation include angiotensin, leptin, interleukin (IL)-1β, -17, -20, C–C motif chemokine ligand (CCL)-2, -3, -5, damage-associated molecular patterns (DAMPs), pathogen-associated molecular patterns, osteopontin and hedgehog ligands.[58] The profibrogenic effects of these molecules are mediated by downstream pathways, including the Smad, Hippo-YAP-TAZ, Erk and MAP kinase pathways.[58] Collectively, these divergent features of HSCs reflect a level of cellular heterogeneity that has only become apparent with the emergence of single-cell analytic techniques.[60]

The production of extracellular matrix (ECM) is considered the primary disease-driving feature of activated HSCs, promoting the formation of fibrotic septa, and disrupting the normal liver's architecture and biomechanical properties.[61,62] Conceptually, the amount of fibrosis present in the liver reflects the balance between pro-fibrogenic and fibrinolytic mechanisms.[61] To date, however, this concept has not been formally validated, but the general assumption is that fibrosis accumulation results from excess profibrogenic activity relative to fibrinolysis.

The architectural changes in advanced fibrosis are associated with parenchymal extinction and severe clinical sequelae including the loss of liver function, the development of portal hypertension and an increased risk of liver cancer.[60,63,64] A set of core genes (*e.g. GAS7, SPON1, SERPINE1, LTBP2, KLF9, EFEMP1*) drive the fibrogenic activation of HSC subclusters, as evidenced from patient biopsies, HSC cultures and rodent models.[65] Activated HSCs produce a wide range of ECM molecules, including type I collagen, the most abundant ECM component of the fibrotic liver,[66–68] other fibrillar and non-fibrillar collagens, along with glycoproteins that include hyaluronan, tenascin, decorin, fibronectin, periostin, lumican and laminins.[69–71] In the early stages of MASH fibrosis, an increase of pericellular fibrosis leads to the characteristic perisinusoidal fibrosis, especially in the pericentral zone (zone 3). In later stages, fibrotic septa and bridging fibrosis may develop.[61] In advanced fibrosis, collagen becomes crosslinked, making it stiffer and more resistant to degradation. This process is initiated by enzymes that include lysyl oxidases,[72] among others.[73] In addition to the replacement of functional parenchyma by ECM, the increased stiffness also impairs the function of hepatocytes. Stiffness leads to a loss of hepatocyte function and dedifferentiation, *e.g.* via the downregulation of transcription factor HNF4α (hepatocyte nuclear factor 4α) and/or upregulation of the YAP/TAZ pathway.[74–76] Moreover, many ECM components signal via specific receptors such as integrins and discoidin domain receptors in resident and non-resident liver cells, affecting a wide range of responses such as proliferation and regeneration, differentiation and inflammation.[69,77,78]

A second characteristic feature of activated HSCs is their increased contractility. αSMA (α-smooth muscle actin) is strongly upregulated during HSC activation and contributes to HSCs' regulation of vascular tone,[63] along with protocadherin 7.[79] At the same time, HSCs may also promote angiogenesis via the secretion of VEGF (vascular endothelial growth factor) and angiopoietin-1.[80,81] Together with the increased stiffness

of the fibrotic liver, angiogenesis and higher HSC contractility promote the development of portal hypertension, a characteristic feature of advanced liver disease, and may contribute to many of its complications, including decreased liver function, variceal bleeding and ascites formation.[82]

Finally, activated HSCs engage in a bidirectional crosstalk with discrete immune cell populations. While immune cells such as macrophages, T cells and B cells contribute to HSC activation in MASH,[30,83] HSCs may regulate hepatic inflammation and immunity by controlling immune cell recruitment and activity in MASH.[84] The secretion of chemokines, including MCP-1 (also known as CCL2), IL-8, RANTES SDF-1/CXCL-12, and the expression of adhesion molecules ICAM-1 and VCAM-1 promote the infiltration of lymphocytes and monocyte-derived macrophages.[85–89] HSCs can modulate immune responses by acting as MHC class II-expressing antigen-presenting cells and by simulating T cells via CD86 expression.[90–92] However, HSCs have limited antigen-presenting capabilities *in vivo* and likely contribute to the tolerogenic environment of the liver through expression of ICAM-1, IDO, PD-L1, retinoic acid and TGFβ, affecting cytotoxic and regulatory T cells, B cells and myeloid-derived suppressor cells.[93–102]

Progressive HSC activation modifies their interactions with other cells, as shown in single cell-based ligand-receptor analysis.[50,51,65] In addition to an overall increase in HSC interactions, there is a major shift in the pattern of interactions. HSC interactions with hepatocytes and LSECs, which maintain homeostasis and epithelial health and are characteristic of qHSCs, decrease during the progression of CLD. In parallel, HSC interactions with inflammatory cell types, cholangiocytes, and LSECs, as well as autocrine HSC-HSC interactions increase during CLD progression. Examples of interactions that decrease with HSC activation include those with hepatocytes, endothelial cells and Kupffer cells, mediated by HSC mediators HGF, RSPO3 and HSC-enriched BMP family members.[31,32,46] One example of increased ligand-receptor interactions includes NTF3-NTRK3. NTF3-NTRK3 this interaction further amplifies the activation and and proliferation of HSC within fibrotic septa, where they are densely packed in proximity to one another and distrant form signals from hepatocyte- and Kupffer cell-derived signals.[34] Together with the concomitant upregulation of type I collagen, αSMA and other ECM components during activation, there is a shift from beneficial, homeostatic HSC interactions toward fibro-pathogenic HSC interactions in liver disease and MASLD progression.[39]

Senescent HSCs

After many years of MASLD, during which HSCs have undergone many rounds of proliferation, they can undergo replicative senescence.[103] Senescence limits their ability to proliferate and produce ECM and can thereby reduce further expansion and fibrosis,[104,105] as well as long-term consequences such as the development of HCC.[106] However, senescent HSCs are also characterised by an increased expression of inflammatory mediators, termed the senescence-associated secretory phenotype (SASP). A recent study has characterised the senescent features of HSCs' SASP in MASH.[103] A unique cluster of molecular markers define senescence in this population, underscoring that each cell type may have different components of a senescence signature. Regardless, SASP of HSCs is thought to provoke inflammation and the recruitment of inflammatory cells, thereby promoting the progression of chronic liver disease.[107] Thus, clearance of senescent HSCs using chimeric antigen receptor (CAR) T cell therapies is a potential therapeutic strategy.[108]

Deactivated HSCs

Chronic liver diseases such as MASLD are characterised by alternating phases of disease progression and regression. During regression, activated HSCs may undergo cell death[109] or revert to a deactivated or inactivated phenotype.[110,111] Deactivated HSCs express a similar transcriptome as qHSCs but retain memory that renders them more prone to activation than their quiescent counterparts.[110,111] Deactivated HSCs have been identified in MASLD.[112] With HSCs constantly cycling between different states, it is possible that deactivated HSCs accumulate over time in chronic liver diseases, including in patients with MASLD.[113] Moreover, deactivation leads to a higher expression of homeostatic and protective HSC mediators, characteristic of qHSCs, which may help to restore liver function,[32] making drivers of HSC deactivation appealing therapeutic targets.[39]

How hepatocytes trigger HSC activation and liver fibrosis

Both animal studies and clinical trials have demonstrated that treating the underlying disease-driving metabolic abnormalities results in an improvement of liver fibrosis in MASLD.[114–120] Therefore, it is important to understand the mechanisms through which hepatocytes trigger HSC activation and liver fibrosis. The initial precipitant of HSC activation in MASLD results from signals emanating from stressed or dying steatotic hepatocytes.[121–124] However, rather than directly causing HSC activation, several key events may need to occur simultaneously (Fig. 1), which engage multicellular networks that involve (i) signals from macrophages, in particular release of TGFβ; (ii) a loss of inhibitory signals from fenestrated LSECs; (iii) activation of latent TGFβ by thrombospondin 1 and integrin alpha V,[125,126] facilitated through decreased expression of ECM1, a potent inhibitor of latent TGFβ activation;[127,128] and (iv) additional signals from CD8+ T cells, intestinal B cells, regulatory T cells and potentially additional immune cell populations.[30,83,84,129]

In MASLD, hepatocyte stress and death can be triggered by a wide range of pathways that are mostly activated in response to altered quantities and qualities of lipids. Saturated free fatty acids, palmitate, the phospholipid lysophosphatidylcholine or cholesterol can promote fibrosis by directly triggering endoplasmic reticulum (ER) stress, profibrogenic signalling pathways, including Hedgehog, YAP/TAZ and NOTCH,[122] or by causing lipotoxic hepatocyte death,[130,131] which subsequently induces inflammation and fibrosis (Fig. 1). The accumulation of lipids leads to ER stress in hepatocytes and activation of the unfolded protein response in animal models and patients with MASLD.[132–134] While the unfolded protein response is initially an adaptive response, ER stress becomes maladaptive, inducing pro-inflammatory signalling pathways such as JNK, NF-kB, NLRP3, and driving the expression of caspase 2, which collectively trigger inflammation, fibrosis and hepatocyte death.[135–137] Hedgehog pathway activation tracks with liver injury and fibrosis in patients with MASLD, and hepatocyte ballooning has also

been linked to the activation of HSCs via the secretion of sonic hedgehog.[138–140] YAP and TAZ expression are strongly increased in hepatocytes in mouse models and in patients with MASLD.[141,142] The upregulation of TAZ is mediated by increased hepatocyte cholesterol via an ASTER-B/C-soluble adenylyl cyclase-RhoA-mediated pathway that suppresses β-TrCP-mediated TAZ degradation.[143] TAZ activation in hepatocytes promotes MASLD-induced liver fibrosis and is mediated by enhanced secretion of profibrogenic mediators including Indian hedgehog.[142] Hepatocyte-specific deletion of YAP reduces carbon tetrachloride-induced liver fibrosis in mice, but the contribution of YAP to MASLD-induced liver fibrosis was not tested in this study.[141] NOTCH activation tracks with MASH severity in patients, and NOTCH loss- and gain-of-function studies in mice underscore hepatocyte NOTCH's activity in promoting liver fibrosis.[144] Through the induction of osteopontin as well as CCL2,[144,145] NOTCH also triggers the expression of EphB2 in hepatocytes in mouse and human MASH.[146] EphB2 promotes inflammation and fibrosis in MASLD as shown by loss- and gain-of-function studies in mice.[146]

Lipid overload can trigger hepatocyte death, including apoptosis, ferroptosis, necroptosis and pyroptosis, and drive disease progression in MASLD.[122,123,147] In mice fed a methionine- and choline-deficient diet, global caspase 3 knockout had no effect on alanine aminotransferase (ALT) levels or NAFLD activity score (NAS), but did reduce fibrosis.[148] GPX-4 (glutathione peroxidase 4) is essential to protect hepatocytes from ferroptosis as shown by constitutive or inducible knockout studies.[149,150] Due to this pronounced effect, most studies on ferroptosis have relied on pharmacologic inhibition of ferroptosis, which improves MASLD and MASLD fibrosis.[151–154] Notably, vitamin E, an antioxidant that blocks ferroptosis,[155] not only extended the life-span of mice with hepatocyte-specific knockout of *Gpx4*, but also improved MASLD in the PIVENS trial.[149,156] Cell death leads to a wide range of signalling pathways that promote inflammation and liver fibrosis in MASLD and other liver diseases.[122,123] These include the recruitment of inflammatory cells, efferocytosis and TGFβ release, as well as the release of DAMPs, such as nuclear DNA, mitochondrial DNA, HMGB1, ATP, UDP-glucose, and apoptotic bodies. DAMPs may act on macrophages and HSCs to trigger fibrogenic signalling cascades (Fig. 1).[157] Examples of DAMPs directly triggering the activation of HSCs include P2Y14 receptor ligands and HMGB1.[122,124,158,159]

In summary, lipid overload elicits a wide range of signalling cascades, the release of profibrogenic mediators, as well as cell death in hepatocytes, which may all serve as therapeutic targets in MASLD.

How macrophages modulate HSC activation, fibrosis and fibrosis regression in MASLD

Macrophages are highly plastic and exert various roles in tissue homeostasis, injury and repair.[160,161] The pivotal role of macrophages in the development and resolution of MASLD make them attractive therapeutic targets. Hepatic macrophages are comprised of distinct subsets with differing origins and functions, including tissue-resident Kupffer cells and infiltrating monocyte-derived macrophages, both of which exhibit remarkable plasticity.[162] Kupffer cells detect hepatocyte stress and injury signals – whether from neighbouring cells or systemic sources – activating inflammatory pathways, recruiting monocytes and other immune cells through chemokine signalling, and clearing cellular debris. Monocyte-derived macrophages contribute significantly to fibrogenesis but also participate in resolving fibrosis (Fig. 3).[163–165]

Profibrogenic actions of macrophages

Liver macrophages with an inflammatory phenotype promote the progression of MASLD, with their accumulation correlating with disease severity in human biopsies.[166] Advances in single-cell RNA sequencing have unveiled an unprecedented level of detail in the heterogeneity of hepatic immune cells, highlighting profound changes in myeloid cells and macrophages during

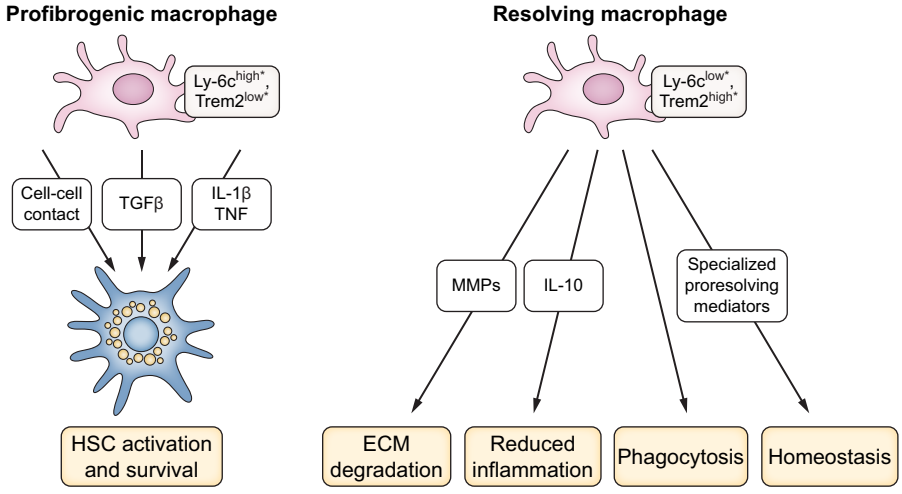

Fig. 3. Macrophage states in MASLD. During MASLD progression, profibrogenic macrophage subsets (*Ly-6c^{high} Trem2^{low} in mice, further characterisation in patients needed) promote HSC activation and survival through the secretion of TGFβ, pro-inflammatory mediators like IL-1β and TNF and through physical contact. During MASLD resolution, specific subsets of macrophages (*Ly-6c^{low} Trem2^{high} in mice, further characterisation in patients needed) degrade ECM via high MMP expression and promote the return to homeostasis, additionally through phagocytes and the secretion of anti-inflammatory and pro-resolving lipid mediators. Shifting the macrophage balance from profibrogenic to pro-resolution may represent a strategy for the treatment of MASLD fibrosis. ECM, extracellular matrix; IL, interleukin-; MASLD, metabolic dysfunction-associated steatotic liver disease; MMPs, matrix metalloproteinases; TGFβ, transforming growth factor β; TNF, tumour necrosis factor.

MASLD progression, helping contextualise findings in a spatial orientation.[167] From a spatial perspective, the occurrence of hepatic "crown-like structures" (*i.e.* macrophages surrounding dead or dying hepatocytes) as well as bile duct-associated macrophages have been linked to MASH with fibrosis progression in mice and humans, and both phenomena relate to infiltrating monocyte-derived macrophages.[168,169] Among Kupffer cell subsets, the CD206^{hi} ESAM^{+} population has been implicated in fatty acid metabolism, potentially driving MASH progression.[170]

Single-cell analyses have underscored the critical role of monocyte- or bone marrow-derived macrophages in MASH. Monocyte-derived macrophages can replace Kupffer cells, while adopting phenotypes such as "lipid-associated macrophages" (LAMs) or "scar-associated macrophages" (SAMs) in MASH, characterised by markers like TREM2, CD9, and osteopontin.[170–172] These hepatic MASH-associated macrophages thereby share many phenotypic markers with LAMs in adipose tissue.[173]

Mouse models have provided foundational insights into the functions of these macrophage subsets. Contrary to initial assumptions, TREM2^{+} macrophages associated with MASH, which were thought to promote inflammation and fibrosis, instead were found to mitigate inflammation and even support fibrosis resolution.[174,175] Interestingly, the resolving phenotype of TREM2+ macrophages in hepatic fibrosis regression applies to both recruited and resident macrophage subsets that cooperate in tissue repair.[176] In addition, the Notch-RBPJ signalling pathway can regulate monocyte differentiation into inflammatory (and fibrogenic) macrophages in MASLD models, with RBPJ deficiency promoting reparative responses.[177] In advanced fibrosis, however, the loss of Kupffer cells and their replacement by monocyte-derived macrophages impair essential homeostatic functions.[178]

Although many findings are based on mouse models, human liver single-cell RNA-seq data have identified SAMs as a distinct population residing within fibrotic niches in cirrhotic livers.[179] Proteo-genomic studies combined with spatial mapping reveal that LAMs (and SAMs) typically localise near intrahepatic bile ducts in healthy conditions, but migrate towards steatotic areas in MASLD, driven by HSC-derived CCL2 chemokine signalling.[180]

Macrophages are considered essential for HSC activation. The genetic or pharmacologic depletion of macrophages has demonstrated a strong reduction of HSC activation and liver fibrosis.[163,181,182] It is likely that the main effects of macrophages on HSC activation and fibrosis result from the release of TGFβ (Fig. 3).[183] In MASH, the release of TGFβ by macrophages requires the macrophage c-mer tyrosine kinase (MerTK) receptor and mice lacking MerTK or humans with hypomorphic MERTK variants are protected from MASH fibrosis.[184] Recent studies suggest that contact-dependent signals between macrophages and fibroblasts create mechanical stress that allows full-blown TGFβ-mediated fibroblast activation in soft environments[185] and may, therefore, be crucial for fibroblast activation in the early stages of MASH, where livers are still mechanically soft. Furthermore, macrophages provide survival signals to HSCs via IL-1β and tumour necrosis factor (TNF).[186] Together, the activation and survival signals significantly contribute to maintaining a pool of activated HSCs that promote fibrosis in MASH. Besides HSCs, macrophages also interact with other immune cells.[168] For instance, in MASH, activated platelets interact with hepatic macrophages, exacerbating inflammation, and indirectly, fibrosis.[187] Intestinal B cells interacting with hepatic macrophages via Fc-receptor γ further amplify metabolic T cell activation and fibrosis in a microbiota- and antigen-independent fashion.[83]

Fibrosis resolution by macrophages

The restorative properties of macrophages make them highly attractive for the treatment of liver fibrosis.[168] Genetic depletion of macrophages has not only revealed a role for macrophages in hepatic fibrogenesis but also during the recovery phase, demonstrating a failure of ECM degradation in the absence of macrophages.[163] Macrophage-expressed matrix metalloproteinases (MMPs), including MMP-9, MMP-12 and MMP-13, constitute major effectors contributing to the degradation of collagen during resolution stages.[40,188,189]

Fibrolytic macrophages are phenotypically distinct from fibrogenic macrophages and are characterised by low expression of Ly-6C(lo), enrichment of Trem2, and an M2-like phenotype in mice.[40,175] Efferocytosis of dead hepatocytes mediates phenotypic shifts in macrophages, including an upregulation of anti-inflammatory IL-10 and pro-resolution lipid mediators, as well as feed-forward loops that increase the phagocytotic and efferocytotic capacity of macrophages (Fig. 3).[124] Moreover, macrophages interact with neutrophils to promote tissue repair.[190] The therapeutic potential of fibrolytic macrophages has been demonstrated in mice and is currently being investigated in patients.[41,191] Beyond their pure fibrolytic actions, Ly-6C(lo) Trem2(hi) macrophages may also exert other restorative functions,[192] consistent with a key role for macrophages in tissue homeostasis.[160]

Open questions about antifibrotic therapies in MASH

Whereas treatment of other chronic liver diseases (*e.g.* chronic HCV infection), have clearly defined endpoints such as viral eradication, MASLD represents a challenge due to the wider range of hepatic and extrahepatic clinical endpoints, including cardiovascular mortality, and its multifactorial pathophysiology that includes genetic and behavioural factors, unique disease subtypes, as well as complex disease-driving interactions between multiple cell types and organs.[1,2,73,193] Several cell types, including hepatocytes, immune cells and HSCs, as well as mechanisms controlling food intake, metabolism and energy expenditure, represent potential therapeutic targets in MASLD.[1,15,194] Moreover, treatment for a disease such as MASLD may require lifelong therapy.

Therapeutic concepts and clinical endpoints

Conceptually, the treatment of MASLD in early stages, where metabolic abnormalities dominate, may differ from more advanced stages, in which fibrosis and parenchymal extinction are characteristic (Fig. 4). Notably, data from recent positive phase III trials show that only 25-37% of patients respond to current MASLD therapies (*vs.* 12-22.5% in the placebo groups) when including fibrosis improvement as an endpoint,[117,195] suggesting the need for individualised or combination therapies to improve response rates. Currently accepted primary endpoints for phase III trials in MASLD constitute: (i) the

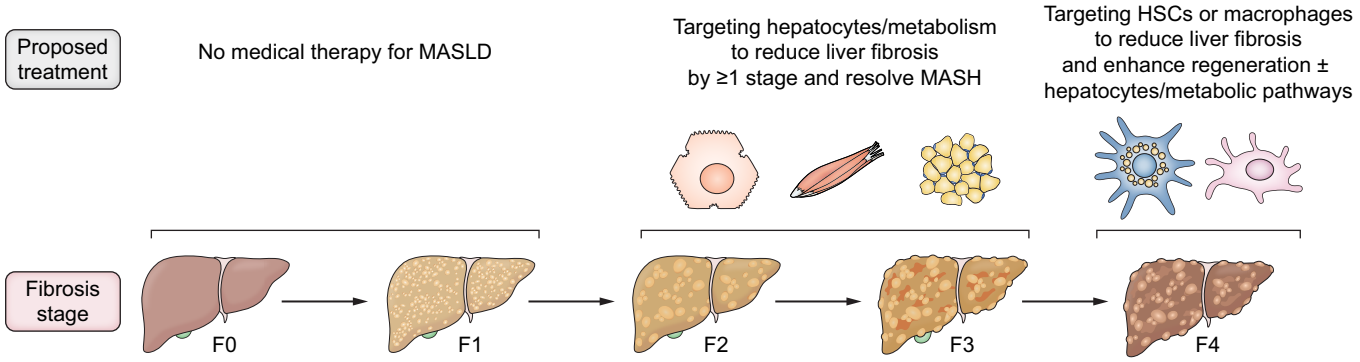

Fig. 4. Stage-specific therapeutic concepts in MASLD. While early stages (F0-F1 fibrosis) may not require medical therapy, encouraging data suggest that hepatocyte- and metabolism-directed therapies may not only improve the underlying metabolic abnormalities but also achieve reversal of fibrosis by ≥1 stage in subsets of patients with F2-F3 fibrosis. In patients with cirrhosis (stage F4), hepatocyte- and metabolism-directed therapies alone seem to have little efficacy in reversing fibrosis. Instead, HSC- and macrophage-directed therapies may be more appropriate for patients with F4 fibrosis, possibly in combination with hepatocyte- and metabolism-directed therapies. HSC, hepatic stellate cell; MASH, metabolic dysfunction-associated steatohepatitis; MASLD, metabolic dysfunction-associated steatotic liver disease.

resolution of steatohepatitis without worsening of fibrosis and (ii) the regression of liver fibrosis by at least one stage without worsening of steatohepatitis.[196] Some trials in patients with advanced fibrosis (*e.g*. F3) also seek to demonstrate lack of progression to cirrhosis rather than regression, which is considered by regulatory agencies as a 'hard endpoint'. In clinical practice, treatments will likely be patient-specific and will need to consider long-term outcomes, which are not only determined by beneficial effects of treatments on MASH resolution and fibrosis improvement but also on cardiovascular mortality (Table 1).

Remarkably, there are no studies linking the level of histologic activity with fibrosis regression; even a potential link between inflammation and fibrosis progression is not well established. Moreover, the extent of inflammation *per se* does not correlate with outcomes in MASH, only fibrosis does.[8] Similarly, there are limited data regarding regional differences in rates of fibrosis regression (*e.g.* septal *vs.* perisinusoidal). A key question is what is the "point of no return" for MASH fibrosis? The limited data available in HCV suggests that regression in HCV following cure is unlikely in the presence of elevated hepatic venous pressure gradient.[197] Nonetheless, a recent study from India suggests that even some patients with decompensated cirrhosis can recompensate following HCV cure.[198] It remains to be determined if similar prospects for regression apply to MASH fibrosis following effective therapies.

Which patients should be treated and for how long?

Patients with MASLD and ≥F2 fibrosis stage or a NAS ≥4 are at the highest risk of progression and hepatic decompensation, and therefore represent the group of patients who will benefit most from current therapies.[12,15] Accordingly, EASL-EASD-EASO Clinical Practice Guidelines recommend that adults with non-cirrhotic MASH and ≥ stage F2 liver fibrosis should be considered for a MASH-targeted treatment with resmetirom, the first FDA-approved treatment for MASH, whereas there are no recommended MASH-targeted pharmacotherapies for the cirrhotic stage.[16]

Beyond the fibrosis stage and NAS, integration of additional criteria such as polygenic risk scores may further identify patients at risk.[199] For example, integrating the polygenic risk score-hepatic fat content, which integrates genetic variants in patatin-like phospholipase domain-containing protein 3 (*PNPLA3*), *TM6SF2, MBOAT7,* and *GCKR*, can further stratify risk in patients with MASLD and may be used to identify individuals that may benefit most from therapeutic interventions.[199,200] Consistent with the loss of steatosis and metabolic alteration and a predominance of ECM accumulation in cirrhosis, most current trials targeting hepatocytes and metabolism are focusing on F2-F3 stages,[118,119,201,202] whereas trials of direct antifibrotics are focusing on F3-F4 stages.[203] However, for MASH-associated compensated cirrhosis (*i.e.* F4 stage), clinical trials are increasingly moving away from histological fibrosis improvement as an endpoint and are instead focusing on non-invasive fibrosis tests (*e.g*. ELF), portal pressure measurement and clinical outcomes.[204]

For all MASLD therapies, the effects on systemic health and overall survival are a key consideration, because mortality in patients with MASLD is significantly driven by cardiovascular events.[205] Recent studies have suggested that MASLD can be divided into two distinct subgroups, presenting either with an aggressive disease limited to the liver, or a more systemic disease associated with a higher risk for cardiometabolic disease.[38,193] While one could speculate that these forms may require distinct treatments, (*e.g*. focusing on the prevention of fibrosis progression in the liver-specific form and the prevention of cardiovascular disease in the cardiometabolic form), links between MASLD, including fibrosis, and cardiovascular health are complex and further studies are warranted. Likewise, it is uncertain how long patients will require treatment for MASLD fibrosis, but most assume it will be lifelong unless disease drivers are mitigated. It can be envisioned that, after achieving fibrosis reduction, therapies could be shifted towards interventions that maintain metabolic health and thereby halt recurrent or progressive MASLD and cardiovascular disease. A recent study, in which patients with compensated liver cirrhosis were treated with FGF21 analogue efruxifermin, suggests that

Table 1. Summary of antifibrotic therapies tested in clinical trials that target hepatocytes, HSCs or macrophages.

Drug class	Clinical development	Fibrosis reduction by ≥1 stage without worsened MASH	MASH improvement without worsening of fibrosis	Effects on cardiometabolic health
Targeting hepatocytes and/or metabolism				
THRβ agonists				
Resmetirom	FDA-approved for patients with MASH and F2-F3 fibrosis (NCT03900429)	24.2% (80 mg) and 25.9% (100 mg) *vs.* 14.2% (placebo) after 52 weeks[117]	25.9% (80 mg) and 29.9% (100 mg) *vs.* 9.7% in placebo[117]	Reduced LDL-C, apoB and TG; no alterations in HbA1c[117,202,225]
VK2809	Phase IIb trial in patients with MASH and F1-F3 fibrosis (NCT04173065)	44%-57% (across doses) *vs.* 34% (placebo) after 52 weeks[206]	63%-75% (across doses) *vs.* 29% (placebo) after 52 weeks[206]	Not reported
FGF19 agonists				
Aldafermin	ALPINE 2/3 in patients with MASH and F2-F3 fibrosis stage (NCT03912532) ALPINE 4 in patients with compensated MASH cirrhosis (NCT04210245)	31% (0.3 mg daily), 15% (1 mg daily), 30% (3 mg daily) *vs.* 19% (placebo) after 24 weeks[207] 16% (1 mg daily) and 20% (3 mg daily) *vs.* 13% (placebo) after 48 weeks[191]	11% (0.3 mg daily), 18%, (1 mg daily), 22% (3 mg daily) *vs.* 6% (placebo) after 24 weeks[207] Not determined[191]	Significantly decreased body weight and serum TG but unaltered HbA1c in 3 mg group[207]
FGF21 analogues				
Efruxifermin	Phase IIb in patients with MASH and F2-F3 fibrosis, completed (NCT04767529) Phase IIb trial in patients with compensated liver cirrhosis (F4 fibrosis; NCT05039450)	39%* (28 mg) and 41%* (50 mg) *vs.* 20% (placebo) at week 24[118] *liver biopsy patients only 18% (28 mg) 19% (50 mg) vs 13% (placebo) at week 36 (primary endpoint); 21% (28 mg) and 29% (50 mg) vs. 11% (placebo) at week 96 (secondary endpoint)[208]	43% (28 mg) and 60% (50 mg) *vs.* 14% in placebo at week 24[118] 42% (28 mg and 50 mg) vs. 13% in placebo at week 96[208]	Reduced body eight, insulin resistance and hyperlipidaemia[118] Great improvement in HDL and non-HDL cholesterol *vs.* placebo[208]
Pegozafermin	Phase IIb in patients with MASH and F2-F3 fibrosis, completed (NCT04929483)	22% (15 mg weekly), 26% (30 mg weekly), 27% (44 mg biweekly) *vs.* 7% (placebo) at week 24[119]	37% (15 mg weekly), 23% (30 mg weekly), 26% (44 mg biweekly) *vs.* 2% (placebo) at week 24[119]	Improved serum TG, and HDL-C[119]
Efimosfermin	Phase IIb in patients with MASH and F2-F3 fibrosis (NCT04880031)	45.2% (300 mg monthly) *vs.* 20.6% (placebo) after 24 weeks[120]	67.7% (300 mg monthly) *vs.* 29.4% (placebo) after 24 weeks[120]	Cardiometabolic data not yet published[120]
FASN inhibitors				
Denifanstat	Phase IIb in patients with F2-F3 fibrosis, completed (NCT04906421)	41% (50 mg daily) *vs.* 18% (placebo) after 52 weeks[209]	26% (50 mg daily) *vs.* 11% (placebo) after 52 weeks[209]	Significant reduction in TG but not in LDL-C (58% of patients were also on statins)
Pan-PPAR agonist				
Lanifibranor	Phase IIb in patients with non-cirrhotic MASH, completed (NCT03008070)	34% (800 mg daily), 48% (1,200 mg daily), *vs.* 29% (placebo) after 6 months[210]	39% (800 mg daily), 49% (1,200 mg daily), *vs.* 22% (placebo) after 6 months[210]	Improved TG, HDL-C, apolipoproteins, insulin, HOMA-IR, HbA1c and diastolic BP, independent of diabetes status[226]
GLP-1RA				
Liraglutide	Phase II (LEAN) trial in patients with MASH and F0-F4 fibrosis stage, completed (NCT01237119)	Non-significant improvement 26% (1.8 mg daily) *vs.* 14% (placebo) at week 48[62]	39% (1.8 mg daily) *vs.* 9% (placebo) MASH resolution at 48 weeks[62]	Weight loss, improved glucose and Hb1Ac, reduced cardiovascular death, myocardial infarction and stroke[208409,227]
Semaglutide	Phase II trial in patients with MASH and F2-3 fibrosis (amended to F1-F3), completed Phase III trial (ESSENCE) in patients with MASH and F2-3 fibrosis, completed (NCT04822181)	49% (0.1 mg daily) 32% (0.2 mg daily), 43% (0.4 mg daily) *vs.* 33% (placebo) at week 72, $p = 0.48$[211] 37% (2.4 mg weekly) *vs.* 22.5% (placebo), interim analysis at week 72[195]	40% (0.1 mg daily) 36% (0.2 mg daily), 59% (0.4 mg) *vs.* 17% (placebo) at week 72[211] 62.9% (2.4 mg weekly) *vs.* 34.1% (placebo), interim analysis at week[195]	Weight loss, improved HbA1c, blood pressure, TG and HDL-C; reduced heart failure, cardiovascular death, myocardial infarction and stroke in obesity with and without diabetes[228,229,230-235]
Dulaglutide	Open label randomised controlled trial in patients with MASLD and type 2 diabetes, completed (NCT03590626)	No biopsy[212]	No biopsy[212]	Weight loss, body weight ($p = 0.011$), decrease in HbA1c ($p = 0.039$) and TG levels[212,236]

(*continued on next page*)

Table 1. (*continued*)

Drug class	Clinical development	Fibrosis reduction by ≥1 stage without worsened MASH	MASH improvement without worsening of fibrosis	Effects on cardiometabolic health
Dual GLP-1/glucagon agonists				
Survodutide	Phase II trial in patients with MASH and F1-F3 fibrosis, completed (NCT04771273)	34% (2.4 mg weekly) 36% (4.8 mg weekly), 34% (6 mg weekly) *vs.* 22% (placebo) at week 48[213]	47% (2.4 mg weekly) 62% (4.8 mg weekly), 43% (6 mg weekly) *vs.* 14% (placebo) at week 48[213]	Decreased LDL-C, TG and HbA1c (significance not evaluated);[213] ongoing evaluation in the Synchronize trials[237,238]
Cotadutide	Phase II trial in patients with MASH and F1-F3 fibrosis, completed (NCT04019561)	No biopsy[214]	No biopsy[214]	Reduced body weight, HbA1c and TG[214,239,240]
Dual GIP/GLP-1 agonists				
Tirzepatide	Phase II trial in patients with MASH and F2-F3 fibrosis, completed (NCT04166773)	55% (5 mg weekly) 51% (10 mg weekly), 51% (15 mg weekly) *vs.* 30% (placebo) at week 48.[215]	44% (5 mg weekly) 56% (10 mg weekly), 62% (15 mg weekly) *vs.* 10% (placebo) at week 48.[215]	Reduced body weight, improved TG, HbA1c and reduced death from cardiovascular causes[215,241,242,243-246]
Triple GIP/glucagon/GLP-1 agonists				
Retatrutide	Phase II study in obese or overweight patients with weight-related complications other than type 2 diabetes, completed (NCT04881760)	No biopsy[216]	No biopsy[216]	Reduction in body weight, TG, LDL-C and HbA1c[216,247,248]
SGLT-2 inhibitors				
Empagliflozin	Phase II study in patients with MASLD without diabetes mellitus, completed (NCT04642261)	Not determined	Not determined Note: Greater reduction in steatosis[224]	Fewer cardiovascular events and death in type 2 diabetes[249]
FXR agonists				
Obitecholic acid	Phase III trial in patients with MASH and F2-F3 fibrosis or F1 fibrosis with additional risk factors (NCT02548351), completed	22.4% (25 mg daily) *vs.* 9.6% (placebo) after 18 months, p <0.0001.[217]	6.5% (25 mg daily) *vs.* 3.5% (placebo) after 18 months, p = 0.093[217]	Elevated LDL-C, decreased HDL-C, increased HOMA-IR[250,217]
Cilofexor	Phase IIb trial in patients with MASH and F3-F4 fibrosis (NCT02548351), completed	12% (30 mg daily) *vs.* 11% (placebo) at week 48[218]	0% (30 mg daily) *vs.* 0% (placebo) at week 48[218]	No changes in serum lipids or HbA1c[218]
ACC inhibitor				
Firsocostat	Phase IIb trial in patients with MASH and F3-F4 fibrosis (NCT02548351), completed	12% (30 mg daily) *vs.* 11% (placebo) at week 48[218]	2.9% (30 mg daily) *vs.* 0% (placebo) at week 48[218]	Increased TG and VLDL-C, no change in HbA1c[218]
Kinase inhibition				
Selonsertib (ASK1 inhibitor)	Phase III in patients with MASH and F3 fibrosis (STELLAR-3, NCT03053050) Phase III trial in patients with MASH and F4 fibrosis (STELLAR-4, NCT03053063)	12% (75 mg weekly), 10% (125 mg weekly) *vs.* 13% (placebo) at week 48 in F3 patients[219] 13% (75 mg weekly), 14% (125 mg weekly) *vs.* 13% (placebo) at week 48 in F4 patients[219]	No effect on MASH resolution	Not reported in detail
Hepatocyte-directed oligonucleotides				
ION224 (DGAT2 antisense)	Phase II trial in patients with MASH and F2-F3 fibrosis (NCT04932512), analysis in F3 subcohort	46.2% (90 mg or 120 mg, monthly) *vs.* 30.8% (placebo) after 51 weeks.[220]	30.8% (90-120 mg) *vs.* 15.4% (placebo)[220]	Improvement in HbA1c
GSK4532990 (HSD17B13 siRNA	Phase IIb study in patients with MASH and F3-F4 fibrosis (NCT05583344), ongoing	No data yet	No data yet	No data

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Table 1. (*continued*)

Drug class	Clinical development	Fibrosis reduction by ≥1 stage without worsened MASH	MASH improvement without worsening of fibrosis	Effects on cardiometabolic health
ARO-HSD (HSD17B13 siRNA)	Phase I/IIa study in healthy volunteers and patients with MASH (NCT04202354), completed	Not evaluated	Not evaluated Note: ALT decreased by 42% in the highest does	No data
ION455/AZD7503 (HSD17B13 ASO)	Phase I study in patients with MASLD or MASH (NCT05560607)	Not evaluated	Not evaluated	No data
ALN-HSD (HSD17B13 siRNA)	Phase II study in patients with MASH and genetic risk factors (NCT05519475), ongoing	No data yet	No data yet	No data yet
JNJ-75220795 (PNPLA3 siRNA)	Phase I study in patients with MASLD (NCT04844450), completed	Not evaluated	Not evaluated	No data
ALN-PNP (PNPLA3 siRNA)	Phase I study in patients with MASLD (NCT05039710) terminated	Not evaluated	Not evaluated	No data
AZD2693 (PNPLA3 ASO)	Phase IIb study in PNPLA3 I148M carriers with F2-F3 MASH (NCT05809934), ongoing	No data yet	No data yet	No data
AMG 609 (PNPLA3-I148M siRNA)	Phase I study in patients with MASLD carrying the PNPLA3 I148M allele (NCT04857606), completed	Not evaluated	Not evaluated	No data
Targeting HSCs and fibrosis **HSC targeting**				
BMS-986263 (HSC-targeted HSP47 siRNA)	Phase II trial in patients with MASH and compensated cirrhosis (NCT04267393), discontinued	Lacking efficacy (data not published)	Not published	Not reported
Reducing ECM stiffness				
Simtuzumab (LoxI2 inhibitor)	Phase IIb trial in patients with MASH and bridging fibrosis (NCT01672866) Phase IIb trial in patients with MASH and compensated cirrhosis (NCT01672879)	33.9% (75 mg weekly), 34.3 % (125 mg weekly) *vs.* 39.1% (placebo) at week 96 in patients with bridging fibrosis 11.8% (200 mg biweekly), 20.3 % (700 mg biweekly) *vs.* 14.7% (placebo) at week 96 in patients with compensated cirrhosis	Not assessed	Not reported
Reducing HSC activation and contraction				
Belapectin (Galectin 3 inhibitor)	Phase IIb trial in patients with MASH cirrhosis NCT02462967) Phase IIb/III trial ongoing (NCT02421094)	No difference in fibrosis or HVPG but improved HVPG in a subgroup without varices[221] Phase IIb/III trial ongoing	Not investigated in NCT02462967 Phase IIb/3 trial ongoing	Not reported
Targeting macrophages **Blocking of chemokine signals**				
Cenicriviroc (CCR2/CCR5 inhibitor)	Phase IIb in patients with MASH and F1-F3 fibrosis (NCT02217475) Phase III in patients with MASH and F2-F3 fibrosis (NCT03028740)	20% (150 mg daily) *vs.* 10.4% (placebo) at year 1 in phase 2b[222] 22.3% (150 mg daily) *vs.* 25.5% (placebo) (placebo)[223]	No effect on MASH resolution	Neutral to cardiometabolic biomarkers
Cell therapy				
Autologous macrophage therapy	Phase I in cirrhosis of any aetiology and MELD score 10-16 and phase II in compensated cirrhosis with MELD score 10-17 (ISRCTN10368050)	Signal of MELD score stabilization/reduction, no fibrosis data and a non-significant reduction in MELD score[42,43]	Not reported	Not reported in detail

ALT, alanine aminotransferase; ASO, antisense oligonucleotide; BP, blood pressure; ECM, extracellular matrix; HSC, hepatic stellate cell; HVPG, hepatic venous pressure gradient; MASH, metabolic dysfunction-associated steatohepatitis; MASLD, metabolic dysfunction-associated steatotic liver disease; MELD, model for end-stage liver disease; siRNA, small-interfering RNA; TG, triglyceride.

prolonged treatment may be required to achieve fibrosis regression in advanced disease stages.[208]

Will direct antifibrotics ever be successful or should metabolic pathways be the primary target?

Targeting disease-driving metabolic abnormalities in hepatocytes or the multi-organ crosstalk that regulates hepatocytes in MASLD likely represent the most efficient and straightforward therapeutic approaches for MASLD as they can reduce fibrosis by improving the underlying disease, as discussed later in this review. To date, direct antifibrotic therapies have not yet demonstrated clinical success. Notably, phase II clinical trials assessing antagonism of lysyl oxidase like 2 (LOXL2)[251] or the collagen chaperone heat shock protein 47 (HSP47) in HSCs (NCT04267393) have not been successful in patients with MASH and F3-F4 fibrosis. However, rapid progress in refining therapeutic targets on HSCs, and an increasing understanding of matrix synthesis and degradation augur well for eventual success. In the following sections, the current state of direct and indirect antifibrotic therapies will be discussed. In this review, direct antifibrotics are considered drugs that target ECM-producing HSCs or ECM synthesis or degradation directly, whereas indirect antifibrotics are those that achieve fibrosis reduction via indirect mechanisms such as alterations in hepatocytes, other metabolically active tissues or macrophages, thereby altering the production or degradation of ECM by other cells.

Direct antifibrotic therapies targeting HSCs in MASH – emerging strategies

The recognition that activated HSCs are the major source of ECM in MASH has led to efforts to either deactivate these cells, clear them, or inhibit specific features to attenuate their fibrogenic activity. While no direct antifibrotics are yet approved for clinical usage, mounting preclinical evidence suggests that such an approach will be effective, especially if combined with therapies to attenuate the upstream metabolic dysregulation associated with MASH, as described in the preceding section. In this section, we review those targets and potential therapies that directly engage HSCs and are either novel, most promising or nearest to advanced clinical trials in patients with MASH. It is not intended to be an exhaustive list, but rather is representative of the diverse mechanisms of action and approaches to target HSCs in MASH.

With the recent discoveries that among activated HSCs there are functionally and genetically distinct subtypes,[31,34,60,112,252,253] efforts to target an antifibrotic molecule to all activated stellate cells may not be as effective as only targeting those subsets that are clearly promoting fibrosis. This more nuanced approach has begun to take root with recent efforts to deplete only a senescent subset of HSCs (see "**Cell therapies to treat fibrosis**"). Still, current strategies assume that most activated HSCs share sufficient common features that make them all viable therapeutic targets, a conclusion borne out by a recent study documenting strong similarities in the activate HSC transcriptome across different aetiologies of liver disease.[254]

TGFβ

The cytokine TGFβ has long been recognised as the most potent signal driving fibrosis in all tissues, and remains the most important antifibrotic target in HSCs and other fibrogenic cell populations.[255] However, its pleiotropic activities, multiple modes of activation and diverse signalling pathways that are cell type- or cell state-specific make it a challenging target. Moreover, systemic antagonism of TGFβ is not safe, because the inhibition of its developmental, antiproliferative, anti-apoptotic, and anti-inflammatory activities can disrupt tissue homeostasis and promote inflamamtion, autoimmunity and cancer.[255–258] Therefore, TGFβ antagonists are sought that antagonise only its fibrogenic activity while preserving other functions. One strategy seeks to inhibit cell surface integrins that contribute to TGFβ activation at the cell membrane, which underlies the promise of using a small molecule (bexotegrast, PLN-74809) that blocks the activity of αvβ1 and αvβ6 in pulmonary fibrosis[259] and primary sclerosing cholangitis (ClinicalTrials.gov ID NCT04480840). As noted, however, mechanisms of TGFβ activation can vary across tissues – this feature may be beneficial by restricting antagonism only to tissues of interest, or detrimental by limiting the scope of inhibition when more than one tissue is fibrotic.

An exciting new approach has leveraged the discovery that latent TGFβ is complexed with different proteins, each of which mediates different activities of the cytokine. Specifically, whereas release of latent TGFβ from either GARP (encoded by *LRRC33*) largely regulates its immunogenic activity,[260,261] its binding to latent TGFβ binding protein (LTBP) controls its fibrogenic activity.[262] With this knowledge, investigators have developed an antibody that only prevents the release of LTBP-bound TGFβ[262] but does not block the release of TGFβ from GARP or LRRC33, thereby inhibiting fibrosis while preserving TGFβ's immunoregulatory and other activities.[263] As proof-of-principle, this antibody attenuates progression of renal fibrosis in two mechanistically distinct mouse models,[263] but no studies in MASH models have been reported yet. However, a recent study also showed a key role for GARP on HSCs.[227]

Another approach to antagonising TGFβ activity distinguishes between the differential fibrogenic activities of the three major TGFβ isoforms, TGFβ1, TGFβ2 and TGFβ3. A recent study indicates that most of TGFβ's fibrogenic activity can be ascribed to TGFβ2 and TGFβ3,[264] whose antagonism avoids the liabilities of inhibiting TGFβ1; this strategy shows promise in systemic sclerosis but has not yet been explored in liver fibrosis.[265]

Cell therapies to treat fibrosis

With increasing knowledge about the unique features of different HSC subtypes, the prospect of deleting specific HSC populations using engineered T cells has emerged. CAR T cells were first developed to treat haematologic malignancies because the neoplasms express unique cell surface markers that are accessible within the circulation. To generate CAR T cells, DNA constructs encoding transmembrane chimeric receptors are transduced into T cells; their general structure includes an antigen binding domain on the cell surface linked to an intracellular domain that activates T cells upon ligand engagement.[266] Based on the antigen binding specificity, these cytolytic T cells can, in principle, target any accessible cell that expresses the cognate receptor recognised by the CAR T cell. There has been rapid progress since the initial development of CAR T cells, both in their targeting efficiency, specificity, safety

and potency, as well as the types of immune cells that can be engineered to express CARs, including regulatory T cells and macrophages.[267–269] Additionally, safety concerns – specifically, a cytokine release syndrome that may occur after CAR T cell administration – are less common through refined treatment regimens and prompt intervention.

CAR T cells are now being developed for a growing number of indications beyond haematologic malignancies, including solid tumours, autoimmunity and, most recently, senescence and fibrosis. Studies have implicated HSCs as drivers of liver injury and inflammation, leading to an effort to identify cell surface markers of senescence in this cell type to target them for CAR T-mediated clearance.

The markers and roles of HSC senescence have been debated, with some studies suggesting that they promote regeneration and limit injury,[105,270] while more recent reports suggest that senescent HSCs are pro-inflammatory, pro-fibrotic and carcinogenic.[271–273] Part of the confusion may arise from varied definitions of the senescence phenotype. Efforts to establish a universal signature of senescence have been elusive, and each tissue and cell type may have a different repertoire of senescence-associated cell receptors, including HSCs.

Recently, the phenotype and ontogeny of senescent HSCs in mouse and human MASH have been extensively characterised, identifying the expression of some canonical senescence markers such as p21, p16, and β-galactosidase activity, as well as other more restricted cell surface receptors such as CD206.[103,274] In this study, RNA pseudotime velocity analysis has established that senescent HSCs are derived from conventional activated HSCs,[103] consistent with the idea that as injury becomes chronic, activated HSCs can progressively acquire senescent features.

A pioneering study combined the knowledge of CAR T production with senescence biology to seek markers of senescence in HSCs.[108] Using an informatics-based approach, the cell surface protein urokinase plasminogen activated receptor (uPAR) was identified as one such marker, and administration of uPAR-directed CAR T cells in a murine model of MASH attenuated fibrosis, cleared senescent cells and improved serum albumin levels.[108] While uPAR expression has traditionally been ascribed to macrophages and neutrophils, the receptor is indeed restricted to HSCs in early murine MASH, with macrophages also expressing this antigen as the disease advances.[103] Whether uPAR is the ideal target for hepatic fibrosis treatment by CAR T cells remains to be established, but its expression on HSCs is lower and less specific than several other cell surface markers, for example CD206.[103] Interestingly, there is also an ongoing trial of the natural flavonoid quercetin and kinase inhibitor dasatinib for MASH (ClinicalTrials.gov ID, NCT05506488), which, when given together, display senolytic activity in adipose tissue and improve metabolic function in old age.[275]

Complementary to the CAR T cell approach to clear senescent HSCs, studies by Epstein and colleagues developed engineered CAR T cells to target only cells expressing fibroblast activating protein 1 (FAP-1), which is a cell surface receptor that marks fibrogenic cells in several tissues, including the heart and joints, among others.[276–279] Administration of CAR T cells that were transduced *ex vivo* reduced fibroblast numbers and fibrosis, and improved cardiac function in a model of chronic cardiac injury.[276] These findings have established a target that does not rely on senescence and is more specific than uPAR.

A remarkably elegant strategy by the same group built upon the conventional CAR T cell approach, instead developing a method of *in vivo* programming of CAR T cells.[280,281] To do so, mRNA designed to programme T cells into CAR T cells is delivered by lipid nanoparticles that target T cells *in vivo*, instructing them to express a CAR directed to FAP on the fibroblast cell surface, yielding similar therapeutic benefit in the heart as conventional CAR T cells. This *in vivo* methodology has at least two distinct advantages. First, therapeutic CAR T-generating nanoparticles can be produced in advance and therefore available immediately as an "off the shelf" technology, greatly expanding their availability beyond only facilities that can generate *ex vivo* CAR T cells onsite. Second, the use of RNA-expressing lipid nanoparticles avoids integration of genetic material into the cell genome, thereby enabling titration of CAR T cell activity and allowing for discontinuation or repeat administration, while avoiding unrestrained HSC clearance. This *in vivo* methodology has also been employed to target FAP-expressing cells in the liver,[282] complemented by studies using FAP imaging to quantify fibrosis.[283] Importantly, transient induction of anti-FAP CAR T cells significantly reduced fibrosis in MASH by depleting pro-fibrogenic HSCs.[282] Moreover, anti-FAP CAR T cell therapy modulates immune cells, endothelial cells and hepatocytes in a non-cell autonomous manner, mitigating inflammation and restoring hepatic homeostasis.[282]

While these reports underscore the potential benefit of selectively depleting HSC populations to reduce fibrosis, their complete elimination is potentially risky. Studies in mice have demonstrated that when 90-99% of HSCs are depleted using either a cell therapy similar to CAR T cells or diphtheria toxins, the livers fail to maintain proliferation and regeneration due to the loss of paracrine signals from HSCs that support hepatocyte replication,[32,48] highlighting the importance of HSCs in maintaining liver homeostasis, as discussed above. The findings indicate that selective clearance of only those HSC populations that promote fibrosis or transient depletion strategies represent more rational approaches than total HSC clearance.

Chemokine and cytokine antagonism

In addition to TGFβ, a number of other growth factors and chemokine targets are being pursued, including IL-11, CCN2, and CCL24.

A growing body of work, primarily from the laboratory of Prof. Stuart Cook in Singapore, has strongly implicated IL-11 as a target for hepatic fibrosis, including MASH. The cytokine has remarkably pleiotropic activity towards epithelial cells and mesenchymal cells across a number of tissues, including the liver, kidney and heart, among others.[267,279,284–287] In the liver, antagonism or knockout of IL-11 attenuates HSC activation and also reduces steatosis and metabolic derangements within hepatocytes in MASH.[288] Thus, a neutralising antibody to IL-11 has significant potential in attenuating its injurious and pro-inflammatory effects, and a phase I trial establishing its safety has been completed, the results of which are awaited (ClincalTrials.gov ID, NCT05658107).

CCL24 is a circulating chemokine produced by epithelial cells and fibroblasts, which binds to its cognate receptor C-C motif chemokine receptor (CCR)3, to promote inflammation,

cell trafficking and fibrosis.[289] Serum levels of CCL24 correlate with severity of fibrosis, which is especially elevated in patients with primary sclerosing cholangitis. CCL24 levels also correlate with stage of disease in systemic sclerosis.[290] A monoclonal antibody to CCL24 is efficacious in several animal models of liver disease,[291] prompting its evaluation in early clinical trials. A completed phase IB trial in patients with MASLD demonstrated good tolerability and improvement in several serum markers of collagen turnover and inflammation.[289] These encouraging results in the liver have established the rationale for continued clinical testing in patients with MASH, and a phase IIa randomised, placebo-controlled trial is underway (ClinicalTrials.gov ID NCT05824156).

HSC-directed oligonucleotide and drug therapies

HSCs express surface markers such as PDGF receptors, retinol binding proteins, mannose-6-phosphate/insulin-like growth factor-II receptor, fibroblast growth factor receptor, integrins, FAP and Fn14 that enable HSC-directed delivery of cargo to ameliorate liver fibrosis and its consequences such as portal hypertension.[292–300] HSC-targeted approaches may enable selective delivery of drugs and oligonucleotides. The latter can be used to silence or activate gene expression via small-interfering RNA (siRNA), self-amplifying RNA and CRISPR-based methods. Based on promising data on HSC-selective delivery of Hsp47 siRNA via vitamin A-coupled liposomes in a rat model of cirrhosis,[294] phase II clinical trials were started in patients with advanced fibrosis or compensated cirrhosis related to eradicated HCV infection or compensated cirrhosis related to MASH. Hsp47 is a collagen chaperone and its inhibition not only alters collagen expression and alignment but also promotes HSC death due to intracellular collagen misfolding.[294] In patients with eradicated HCV infection and F3-F4 fibrosis (NCT03420768), BMS-986263 led to an improvement in fibrosis by ≥1 stage in 17-21% compared to 13% in the placebo group and a reduction of *HSP47* mRNA in most patients in the higher dose group.[203] However, the effects of BMS-986263 on target gene expression were disappointing, showing only a reduction of 5.9% in *HSP47* mRNA and 10.1% in HSP47 protein levels.[203] Notably, the phase II trial of BMS-986263 in patients with compensated MASH cirrhosis was terminated due to a lack of efficacy (NCT04267393). It is possible that the low target gene reduction, possibly due to suboptimal delivery to HSCs, contributed to the low efficacy. Moreover, killing activated HSCs rather than reverting them to their hepatoprotective, quiescent state might have also contributed to the insufficient therapeutic efficacy. Further clinical development of BMS-986263 is uncertain. However, other HSC-targeted delivery systems and cargo have not yet been tested clinically and may hold great promise, especially for patients in F4 fibrosis stage, if efficient delivery and/or siRNA-mediated target gene suppression can be achieved.

Cell surface proteins, matrix modulators and receptors

Belapectin is a novel complex carbohydrate that antagonises galectin-3, which has been implicated in hepatic inflammation, HSC activation and fibrosis.[301,302] Galectin-3 expression increases during HSC activation but has also been associated with expression on macrophages, inflammation and steatosis[303] .[304] In an advanced fibrosis model in mice, belapectin was highly effective and even led to regression of cirrhosis.[221] In patients with MASH cirrhosis, a phase II trial did not show benefit in liver fibrosis or portal hypertension, with the exception of the subgroup of patients without oesophageal varices at baseline, who had significant decreases in the hepatic veinous pressure gradient and fewer new varices.[305] A clinical trial is underway in advanced cirrhosis. This phase IIb/III NAVIGATE study is an ongoing global, adaptive, randomised, placebo-controlled, double-blind trial in patients with portal hypertension (ClinicalTrials.gov ID NCT02421094), based on its efficacy in reducing hepatic venous pressure gradient in a subset of patients with cirrhosis.[306] Belapectin's mechanism of action *in vivo* is not clear, but it could involve inhibiting the vasoconstrictive activity of activated HSCs that otherwise contributes to portal hypertension.

A monoclonal antibody targeting the non-junctional domain of the claudin-1 receptor expressed on hepatocytes is potently antifibrotic in organoids, cell culture and multiple mouse models of hepatic fibrosis.[307] While claudin-1 is not expressed on HSCs, the antibody's efficacy could in part be due to abrogation of hepatocyte–HSC interactions that promote fibrosis. Ongoing clinical studies are anticipated.

Inhibition of LOXL2, an enzyme catalysing the cross linking and stabilisation of fibrillar collagen, has been considered as an attractive target for an inhibitory antibody based on very promising animal studies;[308] however, a clinical trial showed no efficacy in patients with MASH and advanced fibrosis or cirrhosis.[251] Despite this failure, there remains interest in small molecule inhibitors of all LOXL enzymes,[309] which would overcome concerns that the therapeutic antibody was too large to reach the collagen fibrils, whereas a small molecule is not. Moreover, a recent study indicates that in pulmonary fibrosis, the dominant LOXL isotype is LOXL4,[310] suggesting that a pan LOXL inhibitor that antagonises this enzyme might be more effective than inhibiting only LOXL2 in liver fibrosis.

Metabolic modulators

While most metabolic therapies for MASH primarily target steatosis and metabolic dysregulation in hepatocytes, at least four agents also have direct antifibrotic activity towards HSCs *in vivo*: 1) aramchol; 2) a fatty acid synthase inhibitor (denifanstat); 3) an antagonist to *PNPLA3*, and; 4) a structurally engineered fatty acid (icosabutate). Aramchol is an oral fatty acid-bile acid conjugate that reduces liver fat and improves insulin resistance in experimental MASH, which has led to a phase II trial showing good safety and tolerability, as well as efficacy in reducing liver fat and fibrosis in patients with MASH.[311] On a cellular level, incubation of HSCs with aramchol is directly antifibrotic through inhibition of steroyl CoA desaturase-1.[312] A similar inhibitory effect of denifanstat, a fatty acid synthase inhibitor, on steatosis, as well as a direct antifibrotic effect has been demonstrated in cultured HSCs,[209] complementing promising phase II results in MASH.[313] Prior studies have demonstrated the dependence of HSC activation on autophagic degradation of fatty acids,[314] so denifanstat effectively deprives the HSCs of a source of fuel for activation. A structurally engineered fatty acid, isosabutate, is a more potent form of omega-3 fatty acid, which has been developed

to target metabolic, inflammatory and fibrotic pathways in MASH.[315] In immortalised human HSCs (LX-2 cells), the agent is significantly anti-proliferative.[316,317]

A polymorphism in the *PNPLA3* gene (I148M) was the first gene variant linked to the risk of MASH. While most of the underlying biology of this disease-associated variant has been ascribed to its role in hepatocytes, it is also expressed in HSCs, where the variant gene increases fibrogenic activity.[318,319] However, ongoing clinical trials testing the efficacy of PNPLA3 knockdown are focusing on hepatocyte-directed delivery, as discussed in the following sections.

Intracellular targets

A growing body of evidence, much of it from the laboratory of Dr. AM Diehl, has established hedgehog signalling as a potential antifibrotic target. This nuclear transcription factor has pleiotropic roles including in embryonic development and cancer.[320] In the liver, hedgehog signalling in HSCs is profibrotic through multiple pathways and transcriptional targets including *Smo, Ptc, Gli1* and *Gli2*;[321–325] hedgehog signalling also promotes senescence of hepatocytes, which is pro-inflammatory.[326] Crosstalk with other signalling pathways including Notch and Yap further diversifies the range of activities downstream of hedgehog signalling.[323,327] In a study relevant to human MASH, a pathway of TAZ-driven secretion of Indian hedgehog by hepatocytes leads to paracrine activation of HSCs in mouse MASH.[142]

Despite the solid data implicating hedgehog in HSC activation and fibrosis, current clinical inhibitors are restricted to trials across a range of malignancies, especially haematologic cancers and basal cell carcinoma, as tolerability of these drugs for a non-malignant indication is not acceptable. Development of better tolerated inhibitors could refocus interest on hedgehog as a target for MASH fibrosis.[328]

VAP-1

Vascular adhesion protein-1 (VAP-1) is a cell surface glycoprotein molecule primarily expressed by sinusoidal endothelial cells that promotes infiltration and retention of leukocytes in the liver. In addition to promoting adhesion of leukocytes, VAP-1 has amine oxidase activity, catalysing the oxidative deamination of primary amines to generate hydrogen peroxide and aldehydes. Inhibitors of VAP-1 seek to neutralise either its adhesive and/or enzymatic activities. In addition to their effects on endothelial-leukocyte interactions, which may indirectly affect fibrosis, VAP-1 inhibitors also have direct antifibrotic activity towards HSCs.[329,330] Promising results in animal studies have led to a phase I study in MASH, demonstrating adequate safety (ClinicalTrials.gov ID NCT04897594).

Nuclear receptor ligands

Whereas the sole approved drug in MASH, resmetirom, a thyroid hormone receptor-β (THRβ)-selective agonist, has no established direct antifibrotic activity, two other classes of nuclear receptors have some direct action on HSCs. Farnesoid X receptor (FXR) agonists were among the first drug types showing potential benefit in MASH, with reports of direct antifibrotic activity in isolated HSCs and mouse models through synergy with the nuclear receptor peroxisome proliferator-activated receptor (PPAR)γ.[331] However, it is likely that the primary effect of FXR and PPARγ agonists on fibrosis is through their benefit in attenuating hepatocellular injury and dysregulation. Unfortunately, safety concerns about obeticholic acid, an FXR agonist, led the FDA to decline its approval for clinical usage in MASH. In contrast, a pan-PPAR agonist, lanifibranor, has shown promise based on phase II trials, with its PPARγ component potentially contributing to the reduction of fibrosis seen in this trial[226,332] (ClinicalTrials.gov ID NCT03008070). Based on these promising results, a phase III trial is underway in MASH (ClinicalTrials.gov ID NCT04849728).

ASK1

While the primary activity of apoptosis signal-regulating kinase 1 (ASK1) has been focused on hepatocytes, as discussed later, additional direct antifibrotic activity is possible. Pharmacologic ASK1 inhibition can directly reduce HSC activation[333,334] by suppressing profibrogenic pathways, such as p38 and JNK.[335,336]

Indirect antifibrotics targeting hepatocytes and metabolic pathways in MASH – current and emerging therapies

Treatment of the underlying disease usually represents the most effective therapeutic approach. Accordingly, recent clinical trials in MASLD show that targeting metabolic pathways may improve liver steatosis, injury and fibrosis, whereas others affect steatosis without significantly affecting fibrosis. The mechanisms of fibrosis reduction by these therapies have not been investigated in detail, but it is likely that an improvement of metabolic parameters, amongst others, leads to reduced hepatocyte death and less inflammation, thereby decreasing HSC activation and fibrogenesis.

THR agonists

The THR is a nuclear receptor that regulates cell growth and metabolism and exists in two subtypes. THRα plays a key role in regulating heart rate and hypertrophy.[337] In contrast, THRβ is highly enriched in the liver, where it regulates hepatic lipid and carbohydrate metabolism.[338] Therefore, selective THRβ agonists primarily target the liver, inducing hepatic fatty acid oxidation and reducing steatosis and hyperlipidaemia.[339] Resmetirom, a THRβ-selective agonist, is the first approved by the FDA for the treatment of MASH with moderate to advanced fibrosis.[206] In phase III trials, resmetirom not only reduced triglycerides, LDL cholesterol, hepatic fat and the NAS in patients with MASH but also reduced liver stiffness.[117,202] A highly significant reduction of the fibrosis score by stage without worsening of the NAS was achieved in 24.2% of the patients in the 80 mg and 25.9% of those in the 100 mg resmetirom group compared with 14.2% in the placebo group. VK2809, another THRβ agonist, has shown similar effects on the liver, with a reduced liver lipid content at 12 weeks and MASH resolution with no worsening of fibrosis in 69% across different doses (*vs.* 29% for placebo) as well as improvement in fibrosis by ≥1-stage with no worsening of MASH in 51% of

patients across doses (*vs.* 34% for placebo) in a recent phase IIb trial (NCT02927184).[340] Thus, despite the significant effects on fibrosis, it seems that only a subset of patients experience a reduction of liver fibrosis, at least after 1 year of treatment. Further studies are needed to determine why the resolution of MASH does not translate to a reduction in fibrosis in some patients. Ongoing trials are investigating effects on patients with MASLD and compensated cirrhosis.[341]

FGF21 analogues

Fibroblast growth factor (FGF) 21, a hormone-like protein within the FGF superfamily, regulates glucose and lipid metabolism and energy homeostasis in the liver.[201,342] FGF21 expression is induced by the ingestion of large amounts of carbohydrates and fructose, in particular, as well as alcohol and FXR activation, and exerts pleiotropic effects that collaboratively protect hepatocytes from injury. FGF21 actions include the induction of HNF4α, protection from ER stress and apoptosis.[201] Furthermore, FGF21 exerts systemic effects through adipose tissue and other organs that improve MASLD, such as alterations in food intake and metabolism.[201] Notably, FGF21 induces the secretion of adiponectin, an antifibrotic and anti-inflammatory adipokine,[343] from adipose tissue.[344] FGF21 may also exert effects on HSCs and macrophages.[201,345,346] These pleiotropic activities have generated substantial interest in using FGF21 to treat MASLD and other metabolic disorders. The main limitation of using FGF21 is its short half-life of 0.5-2 h, caused by FAP-mediated cleavage,[347] which has led to the development of FGF21 analogues.

FGF21 analogues, including efruxifermin, pegozafermin, and efimosfermin, are being investigated for their therapeutic potential in MASLD and liver fibrosis. Efruxifermin, an Fc-fusion FGF21 analogue, achieved an improvement in histological liver fibrosis by ≥1 stage without worsening of MASH in 39-41% of patients with MASLD and F2 or F3 fibrosis (*vs.* 20% for placebo) in addition to reductions in liver fat, serum ALT and ELF score in the phase IIb HARMONY trial.[118] Pegozafermin, a long-acting glycopegylated FGF21 analogue, led to a ≥1 stage improvement in liver fibrosis without worsening of MASH in 22-27% of patients (*vs.* 7% for placebo) in a phase IIb trial of patients with MASH and F2 or F3 fibrosis.[119] Efimosfermin, a long-acting IgG-FGF21 fusion protein, treatment resulted in fibrosis improvement of ≥1 stage without worsening of MASH in 45% of patients *vs.* 21% in the placebo group.[120] The failure of pegbelfermin, a pegylated FGF21 analogue, to statistically significantly increase the number of patients achieving a ≥1 stage fibrosis improvement without worsening of MASH in the FALCON 1 and FALCON 2 trials in F3 and F4, respectively,[348,349] suggest that the effects of FGF21 analogues may be stronger in patients with earlier stages of fibrosis where metabolic alterations, the primary target of FGF21, dominate. A recent study in patients with compensated liver cirrhosis (stage 4 fibrosis), suggesting an improvement in liver fibrosis without worsening of MASH at 96 weeks but not 36 weeks of efruxifermin treatment, requires further confirmation.[208]

FASN inhibitors

De novo lipogenesis contributes to the development of MASH.[1] Fatty acid synthase (FASN) is a lipogenic enzyme with a key role in *de novo* lipogenesis and the production of palmitate. Accumulation of lipids and toxic lipid moieties including palmitate, in particular, triggers lipotoxicity in hepatocytes, thereby provoking immune cell infiltration, inflammation and fibrogenesis. Accordingly, hepatocyte-specific knockout of FASN improved steatosis but not ALT levels in genetic mouse models of MASLD induced by ob/ob deficiency or melanocortin-4 receptor deficiency.[350] However, in some settings, hepatocyte-specific knockout exacerbated hyperglycaemia in ob/ob mice.[350] The effect of FASN inhibition was first investigated in a 10-day trial in obese patients, which showed reduced *de novo* lipogenesis and serum ALT levels.[351] In a phase IIa trial, the FASN inhibitor denifanstat (previously TVB-2640) reduced liver fat and improved metabolic, pro-inflammatory and fibrotic markers.[352] In a subsequent phase IIb trial, denifanstat led to a fibrosis improvement of ≥1 stage in 41% of patients with MASLD and F2 or F3 fibrosis compared to 18% of patients in the placebo arm, while more frequently leading to an improvement of the NAS by >2 points and resolution of steatohepatitis without worsening of fibrosis compared to placebo.[313]

Pan-PPAR agonists

PPARs are nuclear receptors which regulate the transcription of genes related to metabolism, inflammation, and cell differentiation (see above). Three types of PPARs have been identified: PPARα, PPARβ/δ and PPARγ. PPARs are activated by free fatty acids and their derivatives. The three PPARs are expressed in multiple cells of the liver, including hepatocytes, HSCs, LSECs and Kupffer cells, as well as in adipose tissue. The key role of PPARα in hepatocyte metabolism has been reinforced by the aggravation of MASLD by hepatocyte-specific PPARα knockout.[353,354] Hepatocyte-expressed PPARδ has a role in diurnal hepatic lipogenesis and peripheral fatty acid use.[355] Cell-specific expression of PPARy has revealed roles in multiple liver cell types, including the negative regulation of HSC activation, the promotion of steatosis in hepatocytes and the protection from hepatic inflammation and damage via myeloid-specific expression.[356,357]

The wide range of effects on metabolism, inflammation and fibrosis has raised interest in utilising PPAR agonists for the treatment of MASLD. While PPARα, PPARy, PPAR-δ and dual PPAR-α and PPAR-δ agonists have shown efficacy in a wide range of metabolic diseases, as well as in primary biliary cholangitis,[358,210] the pan-PPAR agonist lanifibranor appears to be the only PPAR-based therapy with substantial efficacy in patients with MASLD.[332] In addition to improving MASH without worsening fibrosis and cardiometabolic health, lanifibranor led to an improvement of ≥1 fibrosis stage without worsening of MASH in 34-48% of patients compared to 29% in the placebo group, but statistical significance was not demonstrated.[332,359]

Incretin mimetics

Incretins are hormones that are released from the enteroendocrine cells in the ileum and colon in response to orally ingested glucose, stimulating insulin secretion more potently than after intravenous injection of the same amount of glucose. Glucose-dependent insulinotropic polypeptide (GIP), produced by intestinal K cells and glucagon-like peptide 1 (GLP-1), produced by intestinal L cells, represent the two most relevant incretins. GLP-1 is derived from proglucagon, which can either be turned into glucagon or GLP-1 in a cell type-specific

manner.[360] GLP-1 not only increases pancreatic insulin secretion but also reduces food intake through effects on gastrointestinal transit and the central nervous system. GIP is derived from its precursor, pro-GIP. Through its receptor, GIP potentiates glucose-induced insulin secretion. Glucagon is released from alpha cells and counteracts many effects of insulin. However, in combination with GLP-1 receptor agonism, glucagon receptor agonism exerts beneficial effects on the liver, such as increased hepatic glycogenolysis and gluconeogenesis, as well as increased fatty acid oxidation and inhibited lipogenesis without its negative insulin-agonistic effects, which appear to be absent in the presence of GLP-1 receptor agonism.[360,361] Additional beneficial effects of glucagon receptor agonism, such as increased lipolysis, thermogenesis and energy expenditure, are mediated by the adipose tissue.[360] Based on these findings, single GLP-1 agonists, as well as combinations of GLP-1, GIP and glucagon receptor agonists, have been investigated for metabolic diseases, including type 2 diabetes mellitus, obesity and MASLD.[362]

GLP-1 receptor agonists

GLP-1 receptor agonists (GLP-1RAs), including liraglutide, semaglutide, and dulaglutide have been evaluated for their antifibrotic activity in patients with MASLD. In multiple phase II trials, liraglutide, semaglutide and dulaglutide treatment for up to 72 weeks decreased body weight, hepatic steatosis and markers of liver injury in patients with MASLD and F1-F3 fibrosis, but did not lead to significant improvements in liver fibrosis of ≥1-stage without worsening of MASH.[212,363,211,364] However, recent retrospective studies in patients with type 2 diabetes have reported a decrease in liver-related outcomes, including cirrhosis, hepatic decompensation and hepatocellular carcinoma with GLP-1RAs compared to other glucose-lowering drugs.[365–369,213] In support of this are first data from the phase III ESSENCE trial (NCT04822181), demonstrating that semaglutide not only induces MASH resolution without worsening of fibrosis (alongside significant reductions of ALT, AST and gamma-glutamyltransferase), but also leads to a significant improvement in liver fibrosis without worsening of MASH in 37% of patients *vs.* 22.5% in the placebo arm based on intention-to-treat analysis.[195]

Dual and triple GLP-1/GIP/glucagon receptor agonists

The dual GLP-1/glucagon receptor agonist survodutide led to an improvement in MASH with no worsening of fibrosis and a decrease in liver fat content in a phase II trial in patients with MASLD and F1-F3 fibrosis.[214] Notably, survodutide ameliorated fibrosis by at least one stage in 34-36% of patients without worsening of MASH compared to 22% in the placebo group.[214] The dual GLP-1/glucagon receptor agonist cotadutide improved non-invasive markers of fibrogenesis, such as Pro-C3, in addition to ameliorating steatosis and serum levels of ALT and AST.[370] Trials of other glucagon receptor–GLP-1 receptor dual agonists, such as SAR425899 and NNC9204-1177, have been halted due to side effects, which may be higher for drugs with high relative activation of the glucagon receptor.[371,372,215] The dual GLP-1/GIP receptor agonist tirzepatide achieved a ≥1 stage improvement in fibrosis in 51-55% of patients *vs.* 30% in the placebo group as well as MASH resolution without worsening of fibrosis.[216] In a phase IIa study, the triple GLP-1/GIP/glucagon agonist retatrutide normalised liver fat content in up to 86% of patients with MASLD and significantly reduced non-invasive markers of fibrosis such as Pro-C3 but not FIB-4 or ELF.[228] Besides their beneficial effect on liver fat, injury and fibrosis, single, dual and triple GLP-1/GIP receptor agonists improve cardiovascular mortality, a key determinant of outcomes in patients with MASLD, with or without type 2 diabetes.[229,208,373,241,242,374] Furthermore, single, dual and triple GLP-1/GIP/glucagon receptor agonists appear to have a good safety profile, eliciting few adverse effects, which are mostly gastrointestinal.[360]

FXR agonists

FXR is a bile acid-activated nuclear transcription factor highly expressed in the liver and the ileum. FXR regulates the synthesis and enterohepatic circulation of bile acids as well as lipid and glucose metabolism. Notably, the downregulation or knockout of FXR was associated with increased steatosis and MASH development in mouse models.[375–377] Some effects of FXR on the liver, such as downregulation of bile acid synthesis, are mediated by FXR-expressing enterocytes in the ileum, which release FGF 15/19, leading to downregulation of CYP7A1 in hepatocytes via FGF receptor 4. Moreover, ileal FXR activation also leads to the release of FGF21, which affects food intake and metabolism via the central nervous system and adipose tissue. In the liver, hepatocytes and HSCs constitute the main FXR-expressing cell types. Hepatocyte and intestinal FXR appear to exert complementary functions through distinct sets of target genes.[378] HSC-specific knockout of FXR has been associated with a decrease in biliary fibrosis.[250] The profound phenotype of FXR knockout mice and its key functions in bile acid, cholesterol and metabolic regulation made FXR a relevant therapeutic target for MASLD. Indeed, the phase II FLINT trial demonstrated strong effects of the FXR agonist obeticholic acid (OCA) on the NAS but also an increase in LDL cholesterol in patients with non-cirrhotic MASLD.[379] This lipid imbalance could be mitigated by the addition of atorvastatin, as shown in the CONTROL trial.[380] In the phase III REGENERATE trial, patients with MASLD and F2-F3 fibrosis were treated with OCA for 72 weeks. The primary endpoint of fibrosis improvement without worsening of MASH was achieved in 18-23% of OCA-treated patients compared to 12% in the placebo group, but the primary endpoint of MASH resolution was not met.[217,381] Thus, OCA may achieve antifibrotic effects in MASLD that are disconnected from its effect on metabolic alterations. Furthermore, more than half of patients experienced pruritus. Several other FXR agonists, mostly non-steroidal, have been investigated in animal models and clinical trials, including PX-104, cilofexor, TERN 101, tropifexor, MET-409 and vonafexor, and have shown substantial improvements in steatohepatitis and fibrosis in mice.[382] However, clinical trials did not clearly demonstrate advantages over OCA, with pruritus remaining a common side effect.[382]

Long-chain omega-3 fatty acids

Long-chain omega-3 fatty acids are involved in multiple pathways of relevance to MASH. Icosabutate, a structurally modified omega-3 fatty acid, led to significant decreases in MASH and fibrosis biomarkers independent of fibrosis stage and

disease severity in a phase IIb clinical trial (NCT04052516). Besides higher rates of MASH resolution without worsening of fibrosis, a ≥2-point decrease in the NAS and improvement in markers of liver injury, inflammation and fibrosis, icosabutate was associated with a significantly higher frequency of ≥1 stage fibrosis improvement (based on both conventional and AI-assisted histopathology) without worsening of MASH, compared to placebo.[383]

SGLT2 inhibitors

Sodium-glucose cotransporter-2 (SGLT2) is expressed in the human kidney, where it promotes the reabsorption of filtered glucose.[384] SGLT2 inhibitors lead to urinary loss of glucose and can thereby improve type 2 diabetes mellitus and decrease complications such as cardiovascular death and stroke.[385] With insulin resistance and type 2 diabetes mellitus closely associated with MASLD, SGLT2 inhibitors, including empagliflozin, dapagliflozin, canagliflozin and ertugliflozin, have been investigated in patients with MASLD in clinical trials.[224,386–390] Moreover, several studies have reported a decrease in liver fibrosis with SGLT2 inhibitors in animal models of MASLD.[391–393]

In clinical trials, SGLT2 inhibitors reduced body weight, improved dyslipidaemia and decreased liver fat content and markers of liver injury, such as ALT and gamma-glutamyltransferase, while they reduced non-invasive measurements of fibrosis, including liver stiffness, FIB-4 and the NAFLD fibrosis score, in some[388,389] but not all studies.[387,390] Importantly, histological fibrosis was not determined in these trials. As glucose transporters and glycolytic enzymes are upregulated in HSCs during their fibrogenic activation, SGLT2 inhibitors may reduce HSC activation and fibrogenesis, although this has not been extensively experimentally demonstrated.[194] In summary, the effects of SGLT2 inhibitors on fibrosis in MASLD remain to be proven.

ASK1 inhibition

ASK1 is a MAP3K (mitogen-activated kinase kinase kinase) that is activated in many cell types by oxidant and cellular stress, and by the response to injury.[333] ASK1 is activated by intracellular TNFα and ER stress and it goes on to activate the P38/JNK pathway, resulting in cell death.[394] Hepatocyte-specific knockout of ASK1 reduced steatosis and inflammation in a high-fat diet mouse model of MASH.[395] Likewise, hepatocyte-specific knockout of Traf6 activated ASK1 in hepatocytes, increasing hepatocyte death and, subsequently, HSC activation and liver fibrosis.[396] There was great enthusiasm following a positive phase II trial[219] of selonsertib, a small molecule ASK-1 inhibitor, yet the findings did not hold up in a larger phase III trial in patients with advanced fibrosis,[397] significantly tempering interest in further development. Still, the central role of ASK-1 in MASH fibrosis is compelling and further efforts to improve the potency and selectivity of ASK-1 inhibitors are ongoing.

ACC inhibition

Acetyl-coenzyme A carboxylase (ACC) catalyses the carboxylation of acetyl-CoA to malonyl-CoA, the rate-limiting and first reaction in *de novo* lipogenesis.[398] ACC1 and ACC2 are highly enriched in hepatocytes. Genetic silencing or pharmacologic inhibition of ACC1 and ACC2 have been shown to reduce hepatic steatosis and improve metabolic parameters.[399,400] Firsocostat is a liver-directed ACC1/ACC2 inhibitor that is a substrate for the hepatic organic anion-transporting polypeptide transporter and thereby achieves high hepatic concentrations.[218] However, firsocostat was not associated with a significant reduction of liver fibrosis stage without worsening of MASH or improvement of MASH without worsening of liver fibrosis in a phase II trial.[401]

Hepatocyte-directed oligonucleotide therapeutics

The ability to deliver specific oligonucleotides to hepatocytes, *e.g.* via N-acetylgalactosamine (GalNAc) conjugation, has created countless possibilities to therapeutically modulate hepatocyte gene expression for therapeutic purposes. Several therapies based on GalNAc-mediated oligonucleotide delivery to hepatocytes are already FDA approved.[402–404] Besides GalNAc, additional bioconjugations as well as lipid nanoparticles and viral vectors can be utilised for specific delivery to the liver and to hepatocytes.[405] Targeting key hepatocyte pathways that contribute to the progression of MASLD and the development of liver fibrosis is an area of intense clinical and preclinical evaluation.

Hepatocyte-directed oligonucleotide therapeutics in clinical trials

Therapies based on antisense oligonucleotide (ASO)- or siRNA-mediated silencing of common drivers of MASLD, including PNPLA3, diacylglycerol acyltransferase 2 (DGAT2) and 17β-hydroxysteroid dehydrogenase 13 (HSD17B13) are currently being evaluated in clinical trials (Table 1). The PNPLA3 I148M allele is associated with MASLD development and progression to fibrosis and cirrhosis.[406,407] Anti-PNPLA3 ASO-GalNAc ameliorated steatohepatitis and fibrosis in a MASLD model in *Pnpla3* I148M knock-in mice.[408] Several phase I and phase II trials studying the effects of siRNA against PNPLA3 (JNJ-75220795, ALN-PNP), ASOs against PNPLA3 (AZD2693) or siRNA against PNPLA3-I148M (AMG 609) in patients with MASLD carrying the PNPLA3 I148M allele have either been completed or are ongoing (NCT04844450, NCT05039710, NCT05809934, NCT056482140), with first analyses suggesting a reduction of liver fat content for JNJ-75220795.[409]

DGAT2 catalyses the final step in the synthesis of triglycerides from diacylglycerol and long-chain fatty acyl-CoAs.[410] The key pathogenic role of triglyceride accumulation in MASLD pathogenesis, as well as the strong effects of DGAT2 ASOs on hepatic steatosis in rodent models of MASLD, support the concept of therapeutic DGAT2 silencing for MASLD treatment.[411–413] However, it should be noted that DGAT2 ASOs aggravated hepatic inflammation and fibrosis in the methionine- and choline-deficient diet model of MASLD, suggesting potentially dichotomous effects mediated by elevated free fatty acids.[412] A phase II clinical trial examining the effects of ASOs against DGAT2 (ION224) in patients with MASH (NCT04932512) demonstrated an improvement in the NAS without worsening fibrosis.[220] Moreover, analysis in a subcohort of patients with MASH and F3 fibrosis suggest an improvement of fibrosis in patients treated with ION224 compared to placebo (46.2% *vs.* 30.8%).[414] HSD17B13 encodes a lipid droplet-associated protein highly expressed in hepatocytes,

which is upregulated in MASLD liver samples.[415–418] In mice, HSD17B13 overexpression triggers fatty liver.[418] Conversely, patients with an HSD17B13 loss-of-function variant show a reduced risk of progressing to MASH and cirrhosis.[419,420] Several phase I and II clinical studies of GalNAc-conjugated siRNAs (ALN-HSD and ARO-HSD) or an ASO (ION455) against HSD17B13 are currently running (NCT05519475, NCT04202354, NCT05143905 and NCT05560607). First data show lowered hepatic HSD17B13 expression and an over 40% decrease in serum ALT level in patients with MASH treated with ARO-HSD.[421] In summary, clinical trials examining hepatocyte-directed oligonucleotides for PNPLA3, DGAT2 and HSD17B13 are promising, but future studies need to determine their effects on liver fibrosis in long-term clinical trials.

Experimental hepatocyte-directed oligonucleotide therapeutics

A wide range of preclinical studies have explored targets in hepatocytes for oligonucleotide-based therapies in MASLD, including STK25, MST3, ADGRF1, PCSK7, nicastrin, MCJ, AEG-1, TAZ, miR-132, miR-22 and miR-33 (reviewed in[405]). Among these, silencing of MST3 and TAZ inhibits MASLD-induced fibrosis in preclinical mouse models. MST3 is a kinase that localises to intracellular lipid droplets in hepatocytes, where it regulates ectopic fat accumulation.[422] MST3 expression correlates with hepatic lipid content, lobular inflammation and ballooning in human MASH[422] and Mst3-targeting ASOs ameliorated liver steatosis, inflammation, fibrosis, and hepatocellular damage in a mouse model of MASL, mediated by suppressed lipogenic gene expression and ACC.[423] The YAP homologue TAZ was increased in MASH livers from mice and patients.[142] Hepatocyte-specific TAZ silencing ameliorated hepatic inflammation, hepatocyte death, and fibrosis in mouse models of MASH, which was mediated by the repression of fibrogenic mediator Indian hedgehog.[142] GalNAc-conjugated TAZ siRNA could prevent and reverse MASH fibrosis in mouse models of MASL.[424]

Combination therapies

Combining drugs with distinct mechanisms of action or targeting distinct cell types may improve response rates.[15] Due to the lack of established antifibrotic drugs, most combinations have sought to target different disease-driving metabolic pathways.[15] In the phase IIa DUET trial (NCT05415722), the combination of TERN-501, a THR-β agonist, with TERN-101, an FXR agonist, reduced both liver fat content and fibro-inflammation in non-cirrhotic MASH but was no more effective than TERN-501 monotherapy based on corrected T1-weighted imaging, as a marker of fibro-inflammation. Combinations of semaglutide, a GLP-1RA, with firsocostat, an FXR agonist, and/or cilofexor, an ACC inhibitor, resulted in further improvement in liver steatosis and serum ALT compared with semaglutide alone, but the combinations showed only trends or no differences based on several fibrosis measures.[425] In the TANDEM study, the combination of tropifexor or cenicriviroc did not result in substantial improvements in ALT or fibrosis stage compared to monotherapy in patients with F2-3 fibrosis.[426] In the ATLAS trial, the combination of the FXR agonist cilofexor with the ACC inhibitor firsocostat suppressed lobular inflammation and ballooning and achieved a non-significant trend toward fibrosis stage improvement.[401]

In summary, further studies are needed to uncover combinations that achieve a more substantial fibrosis reduction in patients with MASLD. Rather than combining drugs with different modes-of-action to alter hepatocyte metabolism, it may be more effective to combine therapies that target different cell types with key roles in MASLD, for example hepatocyte-targeted therapies combined with either direct antifibrotics or therapies that target macrophages or inflammation. Additionally, high throughput screening could unveil effective combinations , even if the mechanisms underlying their synergy are not clear.

Direct and indirect antifibrotic therapies targeting macrophages in MASLD – emerging therapies

Very similar to the fibrogenic activation of HSCs, ameliorating hepatic injury in MASLD by targeting metabolic pathways will also have indirect salutary effects on inflammatory macrophages. Nonetheless, different direct “macrophage targeting” therapies have been explored, but with no clear clinical benefit yet.[223] Because pathogenic liver macrophages in MASH often originate from infiltrating monocytes, the inhibition of their recruitment is a potential therapeutic strategy to reduce liver inflammation. Chemokine receptors CCR2 and CCR5 play central roles in this recruitment process. The dual CCR2/CCR5 inhibitor cenicriviroc, which effectively reduces inflammatory monocyte recruitment to the injured liver, demonstrated promise in preclinical studies and phase II trials but failed to show significant antifibrotic efficacy in a phase III trial.[427–429] Other agents targeting monocyte recruitment, such as inhibitors of CCR2, CCL2, and CCL5, as well as VEGF-neutralising antibodies, are under development but have not yet established clinical efficacy.[194] Another therapeutic approach involves modulating macrophage activation by inhibiting responses to pathogen-associated molecular patterns, DAMPs, or Toll-like receptors. As noted above, while the ASK-1 inhibitor selonsertib did not demonstrate sufficient antifibrotic effects in a large clinical trial[397] next-generation ASK-1 antagonists are still undergoing evaluation. Targets such as NLRP3 inflammasome activation and ASK-1 have also been explored.[183]

Future therapeutic strategies may aim to shift macrophages from a pro-inflammatory to a restorative phenotype. This approach leverages the inherent plasticity of macrophages to facilitate their transition from an inflammation-inducing state to one that promotes tissue repair and exhibits direct antifibrotic properties.[430] Emerging treatments targeting macrophage metabolism, such as inhibiting glycolysis or fatty acid oxidation pathways, have the potential to drive this phenotypic shift.[26]

A proof-of-concept study and a recent phase 2 open-label randomized controlled trial have demonstrated the safety, feasibility and potential efficacy of cell therapy using *ex vivo*-matured autologous monocyte-derived macrophages, delivered via peripheral infusion, in patients with cirrhosis.[42,43] In preclinical models, cell therapy with alternatively activated macrophages resolved necrosis following acute liver injury, and even ameliorated fibrosis.[41,225] Furthermore, the development of CAR macrophages – with the ability to mount antifibrotic T cell immunity – has been effective in reducing experimental fibrosis (see “Cell therapies in fibrosis”, above).[269] These advances in macrophage reprogramming offer promising avenues for reversing liver fibrosis and supporting liver regeneration, paving the way for novel treatments for chronic liver diseases.

Summary and outlook

Deeper understanding of its pathophysiology, combined with strong collaborative efforts between the research community, regulatory agencies and industry, has led to an expanding landscape of clinical trials in MASLD. As might be expected, treatments that correct the disease-driving underlying metabolic derangements rather than late-stage sequelae, such as HSC activation and fibrosis, appear at present to be the most effective in improving MASH, including fibrosis. At the same time, FDA-approved treatments such as the THRβ agonist resmetirom and promising therapies, such as incretin mimetics, FGF21 analogues and pan-PPAR agonists, ameliorate fibrosis only in some patients and efficacy has not been demonstrated in the F4 fibrosis stage. Thus, development of effective anti-fibrotic drugs, as well as combination or sequential therapies, remains a high priority. Recent breakthroughs in MASLD therapies signal just the beginning of the path towards therapeutic success.

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Abbreviations

ACC, acetyl-coenzyme A carboxylase; ALT, alanine aminotransferase; ASO, antisense oligonucleotide; ASK1, apoptosis signal-regulating kinase 1; AST, aspartate aminotransferase; BMP, bone morphogenic protein; CAR, chimeric antigen receptor; CCL, C–C motif chemokine ligand; CCR, C-C motif chemokine receptor; DAMPs, damage-associated molecular patterns; DGAT2, diacylglycerol acyltransferase 2; ECM, extracellular matrix; ELF, enhanced liver fibrosis; ER, endoplasmic reticulum; FAP(-1), fibroblast activating protein(-1); FASN, fatty acid synthase; FGF, fibroblast growth factor; FXR, farnesoid X receptor; GalNAc, N-acetylgalactosamine; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide 1; GLP-1RA, GLP-1 receptor agonists; HCC, hepatocellular carcinoma; HGF, hepatocyte growth factor; (q)HSCs, (quiescent) hepatic stellate cells; HSD17B13, 17β-hydroxysteroid dehydrogenase 13; HSP47, heat shock protein 47; IL, interleukin-; KCs, Kupffer cells; LAMs, lipid-associated macrophages; LOXL2, lysyl oxidase like 2; LSECs, liver sinusoidal endothelial cells; LTBP, latent TGFβ binding protein; MASH, metabolic dysfunction-associated steatohepatitis; MASLD, metabolic dysfunction-associated steatotic liver disease; MMPs, matrix metalloproteinases; NAS, NAFLD activity score; NTF3, neurotrophin-3; OCA, obeticholic acid; PDGF, platelet-derived growth factor; PNPLA3, patatin-like phospholipase domain-containing protein 3; PPAR, peroxisome proliferator-activated receptor; RSPO3, R-spondin 3; SAMs, scar-associated macrophages; SASP, senescence-associated secretory phenotype; SGLT2, sodium-glucose cotransporter-2; siRNA, small-interfering RNA; TAZ, TAZ, transcriptional co-activator with a PDZ-binding motif; TGF-β, transforming growth factor-β; THR, thyroid hormone receptor; TNF, tumour necrosis factor; uPAR, urokinase plasminogen activated receptor; VAP-1, vascular adhesion protein-1; YAP, Yes-associated protein.

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Conflicts of interest

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Authors' contributions

R.F.S., F.T., A.S. and S.L.F. conceptualized, searched the literature and drafted the manuscript, R.F.S. generated figures and tables.

Supplementary data

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